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UNIVERSITY OF ALBERTA

CARDIOVASCULAR INTERACTIONS BETWEEN ADENOSINE AND NUCLEOSIDE TRANSPORT INHIBITORS

BY

HONG-JUN CHEN

©

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of Master of Science.

DEPARTMENT OF PHARMACOLOGY

Edmonton, Alberta

Spring, 1992

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A.S. Clanachan

A.R.P. Paterson

B. A. Finegan

Date Dec. 18, 1991

TO MY HUSBAND, Dr. LEI DING

WITHOUT WHOM I WOULDN'T BE ABLE TO FINISH THIS THESIS SO EARLY

TO MY TWO LOVELY DAUGHTERS, CATHERINE AND JULIA DING

WITHOUT WHOM I WOULDN'T BE ABLE TO FINISH THIS THESIS SO LATE

ABSTRACT

The endogenous nucleoside, adenosine, has effects on the cardiovascular system and elswhere. These effects are terminated through uptake into cells by nucleoside transport (NT) systems. Inhibition of NT systems potentiate many of the effects of adenosine.

The objectives of this study were to compare several NTIs (dipyridamole, dilazep, NBMPR-P, NBTGR-P and NBdAdo-P) with respect to their effects on arterial blood pressure (BP) and heart rate (HR) and their ability to potentiate and prolong adenosine-induced changes in BP and HR.

All the NTIs (except NBdAdo-P) tested in this study caused varies degrees of depression of BP and HR in rats. In contrast, hypotension and tachycardia was observed in conscious rabbits. The order of potency of NTIs as hypotensive agents in rats is as follows: dilazep>NBTGR-P=NBMPR-P>dipyridamole>NBdAdo-P. Also, they all caused varies degrees of potentiation of adenosine-induced alterations of BP and HR which is an indication of their NT inhibitory effects. The doses of each of the NTIs that cause a two-fold increase in the duration of action of adenosine shows a different order of potency. Therefore, there was a lack of relationship between the hypotensive and the transport inhibitory effects of NTIs. This suggests that adenosine potentiation, that can be obtained with NTIs, may not necessarily be accompanied by undesirable hypotensive actions.

Of all the NTIs tested, NBdAdo-P caused the greatest degree of adenosine potentiation and least amount of hypotension. This compound should be evaluated further as it may serve as a useful agent to alter the disposition of endogenous or exogenous nucleosides without undesirable hypotensive actions.

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I INTRODUCTION

Adenosine is an endogenous nucleoside that is present in all cells. It has regulatory effects on many physiological processes in the body. In the cardiovascular system, it affects both cardiac and vascular functions. It is also involved in regulating neuronal activity, respiration, platelet aggregation and lipolysis. The physiological actions of adenosine are believed to be mediated through membrane-located receptors. Adenosine crosses cell membranes by membrane-located facilitated diffusion systems that possess a broad specificity for nucleosides as well as by sodium-dependent, active transport systems. Passive diffusion is in a minor route for the trans-membrane flux of adenosine and other physiological nucleosides. These nucleoside transport (NT) systems probably play a key role in the control of adenosine concentrations in the vicinity of adenosine receptors as transport, and subsequent intracellular metabolism, are believed to be the major mechanisms of clearance of adenosine from the extracellular space. Therefore, NT inhibitors (NTIs) are expected to potentiate the effects of adenosine.

This chapter reviews the literature that is relevant to the present research. It begins with an overview of the physiological and pharmacological actions of adenosine with emphasis on the cardiovascular system. That is followed by a brief description of adenosine receptors, NT systems and NTIs. Also, the clinical uses of adenosine in cardiovascular disease and the clinical uses of NTIs in chemotherapy are addressed. Finally, a rationale for this research is presented.

A. The physiology and pharmacology of adenosine

Adenosine is considered to be a regulator of many physiological

functions in the body with a wide variety of effects on the cardiovascular system. The basic effects of adenosine on the cardiovascular system were studied in 1929 by Drury and Szent-Gyorgy. They reported that adenosine delayed atrioventricular (AV) conduction by observing its ability to terminate atrial fibrillation in anaesthetized dogs. This was the first description of the electrophysiological effects of adenosine on the mammalian heart. They also reported the effects of slowed sinus rate and increased coronary blood flow after adenosine administration. It was not until 1963, when Berne proposed the hypothesis that adenosine is a local regulator of blood flow (Berne, 1963) that extensive investigation into the regulatory functions of adenosine was begun.

Heart

Adenosine produces a negative chronotropic effect in heart by a direct effect on the sinoatrial (SA) node (Drury and Szent-Gyorgyi, 1929; James, 1965; Chiba, 1972). It has been demonstrated (West and Belardinelli, 1985) that this is due to a shift in the leading pacemaker site in the right atrium, i.e. in the presence of adenosine, the cells adjacent to the SA nodal cells activate first, then the SA nodal cells rather than the other way around.

Adenosine also depresses AV nodal conduction (Drury and Szent-Gyorgyi, 1929). The mechanism of this action has been investigated by Belardinelli and his colleages (1980). They discovered that the conduction velocity from atria to the bundle of His is decreased, but that from the His bundle to the ventricles it is unchanged.

Adenosine also inhibits the force of contraction (Urthaler et al., 1981; Evans et al., 1982) of both atrial and ventricular myocardium. The mechanisms of this action of adenosine differ between atrial and ventricular myocytes and

consists of a direct effect on atria and an indirect effect on ventricular myocardium. In atrial myocardium, adenosine decreases the duration of action potentials and causes a faster repolarization of the myocytes (Belardinelli and Isenberg, 1983). The decreased action potential duration and associated shortened plateau phase causes an attenuated calcium slow current. Therefore, a lower level of intracellular calcium is available for contraction and so causes a negative inotropic effect. In ventricular myocardium, an antiadrenergic effect of adenosine contributes to its inhibitory effects on cardiovascular function (Dobson et al., 1984; Mullane and Williams, 1990). By inhibiting transmitter release at presynaptic sites (Fredholm, 1988), adenosine antagonises ß-adrenoceptor-induced cardiac stimulation, a response mediated via increased cAMP formation (Schrader et al., 1977; Dobson, 1978; Dobson and Fenton, 1983). The increased cAMP stimulates cAMP-dependent kinase, which is necessary for Ca^{2+} channel phosphorylation, and so increases Ca^{2+} available for contraction. More importantly, adenosine, through activation of A_1 receptors, modulates the G-protein transduction mechanisms associated with ß-adrenoceptor activation (Mullane and Williams, 1990). By "uncoupling" the ß-adrenoceptor from adenylate cyclase, adenosine decreases cAMP concentrations and so reduces the force of contraction.

Vasculature

Adenosine produces vasodilation in most vascular beds (Berne, 1986); an exception is the renal vasculature where adenosine causes vasoconstriction (Spielman et al., 1987). The role of adenosine in the autoregulation of the coronary circulation has been well documented (Berne, 1963; Berne, 1980). Under conditions of myocardial hypoxia, such as high metabolic activity or low

oxygen availability, adenosine is released from myocardial cells (Su, 1975; Dewitt et al, 1983) resulting in coronary vasodilation and an increase in blood flow and oxygen supply to the heart. In contrast, under the conditions of excess oxygen supply, such as following nitroglycerin administration (Berne et al., 1983), adenosine formation is reduced.

Adenosine-induced autoregulation of cerebral blood flow has also been proposed (Kuschinsky, 1983; Berne et al., 1983; Phillis et al., 1984). When a decreased oxygen supply or increased oxygen demand occurs in response to ischaemia (Winn et al., 1979) or seizures (Winn et al., 1980), adenosine is released and cerebral blood flow is increased. Adenosine also acts as a metabolic regulator in skeletal muscle by eliciting reactive hyperaemia via inhibition of arteriolar sympathetic tone (Fugslang and Crone, 1987).

Other organs in which adenosine may be involved in the local regulation of blood flow are skeletal muscle (Berne et al., 1983), adipose tissue (Sollevi and Fredholm, 1981), intestine (Granger and Norris, 1980), spleen (Schutz et al., 1983) and kidney (Spielman and Thompson, 1982; Spielman et al., 1987).

The vasodilatory effects of adenosine are believed due to a action of adenosine on vascular endothelial cells and smooth muscle cells through adenosine A_2 receptors.

Neuronal activity

Adenosine is also involved in the control of neuronal activity. Generally, it has a depressant effect on neurons (Kostopoulis and Phillis, 1977; Phillis et al., 1984). It inhibits peripheral neurotransmission (Hedqvist et al., 1978; Hom and Lokhandwala, 1981; Lokhandwala, 1979) by inhibition of the presynaptic release of noradrenaline (Clanachan et al., 1977; Phillis and Wu, 1981; Fredholm et al., 1983; Jonzon and Fredholm, 1984) and acetylcholine

(Ginsborg and Hirst, 1972; Phillis and Wu, 1981) from nerve terminals. In the central nervous system, adenosine also reduces the release of transmitters and acts as an inhibitory modulator of neuronal activity (Stone, 1981).

Other physiological effects of adenosine include inhibition of platelet aggregation (Cusack and Hourani, 1981), inhibition of lipolysis (Fain, 1973; Fredholm and Sollevi, 1985) and centrally mediated inhibition of respiration. Adenosine enhances histamine release (Marquardt et al., 1978) from mast cells by increasing their Ca^{2+} sensitivity (Lohse et al., 1988) and inhibits renin release from kidney (Osswald, 1983; Churchill and Bidani, 1990).

B. Adenosine receptors

The actions of adenosine are mediated via receptors on the extracellular surface of cells. There are at least two main classes of membrane-bound adenosine receptors; these have been named A_1 and A_2. The original classification of adenosine receptors was made by Londos and Wolff (1977). They classified adenosine receptors in various tissues and cell types on the basis of their interaction with adenylate cyclase and the stereochemical specificity of agonists. They proposed two types of adenylate cyclase-associated, adenosine-sensitive sites: the extracellular R-site and the intracellular P-site. Two subtypes of the R-site, A_1 and A_2, cause inhibition and stimulation of adenylate cyclase, respectively (Londos et al., 1980). Adenosine receptor antagonists such as aminophylline, theophylline and caffeine, inhibit the action of adenosine at A_1 and A_2 receptor sites, but not at the intracellular P-site.

In another scheme, extracellular adenosine receptor subtypes are differentiated on the basis of the relative potencies of adenosine and

adenosine analogues on adenylate cyclase activity (Van Calker et al., 1979). A_1 receptors are defined as sites at which the N^6-substituted analogue, N^6-phenylisopropyladenosine (L-PIA) is more potent than adenosine, which is more potent than the amide-substituted 5'-carboxyl analogue, 5'-N-ethylcarboxamideadenosine (NECA). The A_2 receptors show the reverse order of potencies : NECA > adenosine > PIA. The L-stereoisomer of PIA has been shown to be up to 500-fold more potent than the D-stereoisomer for the A_1 receptor; a less than 10-fold potency difference exists at A_2 receptors. In addition, the A_1 receptor is inhibitory to adenylate cyclase which is often linked to a decrease in cAMP and is a high affinity class of adenosine receptor which is activated by low nanomolar concentrations of adenosine. It is present in adipocytes, heart, brain (Evans et al., 1982) and on adrenergic and cholinergic nerve terminals (Collis, 1983; Brown and Collis, 1983). The A_2 receptor often stimulates adenylate cyclase activity that causes an increase in cAMP. It shows a low micromolar sensitivity to adenosine. It has been identified in tracheal (Brown and Collis, 1982) and vascular smooth muscle (Collis and Brown, 1983). Evidence for subtypes of adenosine A_2 receptors has been presented (Bruns, 1987). It is not clear whether both types of receptors (A_1 and A_2) are present in the same cell type.

The intracellular P-site is not really a receptor site (Londos et al., 1983), but a third adenosine-sensitive inhibitory site associated with adenylate cyclase. Its physiological significance is unknown and it has low micromolar affinity for adenosine and analogues.

Most receptor antagonists, e.g. methylxanthines such as theophylline, have not been useful in the study of receptor subtypes as they antagonize both A_1 and A_2 receptors. New compounds that are selective for each receptor

subtype have been developed. Many specific A_1 receptor antagonists, such as 8-cyclopenyl-1,3-dipropylxanthine have been described recently (Lee and Reddington, 1986; Lohse et al., 1987; Bruns et al., 1987).

C. Clinical and possible clinical uses of adenosine

Particular attention has been made over the years toward adenosine actions which might lead to clinical applications. Preeminent among them, has been the cardiovascular effects of adenosine which lead to cardiac depression, vasodilation and hypotension. More recently a cardioprotective effect of adenosine after prolonged cardiac ischaemia has been recognized (Olafsson et al., 1987; Babbit et al., 1989; Gruber et al., 1989; Ledingham et al., 1990).

The cardiac electrophysiological effects of adenosine makes it useful both as a diagnostic and therapeutic agent in patients with cardiac dysrhythmia. Bolus injection of adenosine has been demonstrated to be useful in the acute treatment of supraventricular tachycardia when the reentry mechanism involves the AV node. The efficacy of adenosine is due to the production of transient, high-grade, AV nodal block (Dimarco et al., 1983; 1985). The rapid onset, short duration of action and low incidence of side effects of adenosine contribute to its usefulness. Increasing doses of adenosine can be given until the desired effect is produced. When supraventricular tachycardia is not due to the AV node reentry, such as intraatrial reentry, atrial flutter or fibrillation, adenosine can be used as a diagnostic tool, since in this case adenosine does not terminate the dysrhythmia, but it shows clear atrial activity due to its AV nodal blocking effects.

Continuous infusions of adenosine have been demonstrated useful in inducing vasodilation and controlled hypotension during cerebral aneurysm surgery (Sollevi et al., 1984a). It has potential advantages over other vasodilators such as sodium nitroprusside (SNP) and glyceryl trinitrate (GTN).

Since adenosine has a greater effect on arteriolar resistance vessels than on the venous vasculature, the right and left heart filling pressures and cardiac output are maintained. In addition, adenosine is relatively nontoxic because it is a naturally occurring substance and the hypotension induced by adenosine is easily controlled and reversible due to the rapid inactivation of adenosine in the circulation. There is no tachyphylaxis because adenosine inhibits renin release and noradrenergic neurotransmission, thus preventing reflex increases in arterial pressure. The development of specific adenosine A_2 receptor agonists, such as CI936 (Hamilton et al., 1987) and CGS21680 (Hutchison et al., 1989) makes it possible that hypotensive agents can be found that possess only the beneficial vasodilatory effects of adenosine while having no bradycardic and cardiac depressant actions.

Recent evidence suggests that intracoronary administration of adenosine after prolonged ischaemia can attenuate the prolonged ventricular dysfunction of viable myocytes and limit the vascular injury caused by reperfusion (Babbitt et al., 1989). The mechanisms of this cardioprotective action of adenosine are not yet known but this property of adenosine is important when one considers the high incidence of reperfusion that occurs in the clinical setting.

The antiaggregatory effects of adenosine make it useful in the preservation of platelets in the extracorporeal circulation during cardiopulmonary bypass surgery (Sollevi et al., 1987) and dialysis (Sollevi, 1986).

Other possible clinical uses of adenosine include afterload reduction in low cardiac output conditions caused by high peripheral resistance (Sollevi et al., 1987) and to dilate coronary vessels in some forms of coronary artery disease (Sollevi, 1986).

D. Nucleoside transport systems

It has long been recognized that adenosine and other physiological nucleosides are transported through biological membranes in mammalian cells by specific NT systems. This is supported by the fact that NT is not affected by free bases and sugars but is inhibited by a variety of purine and pyrimidine nucleoside analogues (Oliver and Paterson, 1971). NT systems play an important role in adenosine metabolism, through which adenosine is rapidly taken up into tissues, eg. by endothelial cells (Gorman et al., 1986) and red cells (Moser et al., 1989) and followed by subsequent metabolism. The relative role of these two cell types may vary among different species (Balwierczak et al., 1989). NT systems are not only responsible for the transport of adenosine and other nucleosides from extracellular to intracellular sites but may also play a role in the transport of adenosine from intracellular to extracellular compartments to initiate endogenous adenosine-mediated events (Clanachan et al., 1987).

Since the 1970's, NT systems have been extensively studied in human erythrocytes and other cultured mammalian cells. There are three main types of processes that are responsible for trans-membrane flux of nucleosides: passive diffusion, facilitated difffusion and sodium gradient-dependent transport.

Facilitated diffusion systems have been mostly investigated in mammalian erythrocytes, especially human erythrocytes. Kinetic studies have characterized the facilitated diffusion transport systems as simple carrier mechanisms which are non-concentrative and reversible (Jarvis et al., 1983; Paterson et al., 1983). They have broad specificity for both the physiological ribosides and deoxyribosides and nucleoside analogues (Cass and Paterson,

1972; Jarvis et al., 1983).

Concentrative, sodium-dependent transporters have been reported in intestinal epithelial cells (Jakobs and Paterson, 1986), kidney brush-border vesicles (Le Hir and Dubach, 1984), and spleen (Darnowski et al., 1987).

These NT system are influenced by several physiological conditions such as temperature, pH, and are competitively inhibited by a number of purine and pyrimidine nucleosides (Oliver and Paterson, 1971; Wohlhueter et al., 1979; Kolassa et al., 1978).

E. Inhibitors of NT systems

Facilitated diffusion NT can be inhibited by a number of drugs, the so-called nucleoside transport inhibitors (NTIs). Since adenosine metabolism occurs mainly intracellularly, after transport via NT systems from extracellular sites, NTIs would be expected to potentiate the effects of both exogenous and endogenous adenosine.

Two main group of NTIs are (1) coronary vasodilators and (2) 6-thiopurine nucleoside derivatives. Also, some benzodiazepines such as diazepam, have been recognized as NTIs, and some of the clinical effects of benzodiazepines have been suggested to occur via inhibition of adenosine uptake and the consequent potentiation of adenosine effects (Wu et al., 1981).

The coronary vasodilators, such as dipyridamole, dilazep, hexobendine, and lidoflazine, inhibit NT systems. This group was the earliest recognized NTIs although they are structurally unrelated to purines. Among them, dipyridamole and dilazep are the most extensively studied agents. Many effects of adenosine can be potentiated by dipyridamole, which include adenosine-induced increases in coronary blood flow (Kinsella et al., 1962; Afonso, 1970; Feldman

et al., 1981), negative chronotropic and inotropic actions (Stafford, 1966; Hopkins, 1973), smooth muscle relaxation (Stafford, 1966; Davies et al., 1982) and inhibition nerve cell firing rates (Phillis et al., 1979). The NT inhibitory effect of dipyridamole has been demonstrated in different cell types and tissues which include erythrocytes (Roos and Pfleger, 1972; Jarvis et al., 1982a), platelets (Lips et al., 1980), cardiac tissue (Kolassa et al., 1970; Mustafa, 1979) and cortical synaptosomes (Bender et al., 1980; Barberis et al., 1981). Dilazep has also been shown to enhance the coronary vasodilatory (Raberger and Kraupp, 1971) and the negative inotropic and chronotropic effects of adenosine (Fujita et al., 1980). The adenosine uptake inhibitory effects of dipyridamole have been shown in erythrocytes (Turnheim et al., 1978) and cardiac tissues (Mustafa, 1979).

The 6-thiopurine nucleoside derivatives such as nitrobenzylthioinosine (NBMPR) are also potent NTIs (Paterson et al., 1983). Among them, NBMPR and its water-soluble prodrug nitrobenzylthioinosine 5'-monophosphate (NBMPR-P), have been the most extensively studied. Some homologues of NBMPR, such as nitrobenzylthioguanosine (NBTGR) and N-substituted adenine nucleosides nitrobenzyldeoxyadenosine (NBdAdo) are also potent NTIs as demonstrated by their ability to inhibit nucleoside uptake or NBMPR binding (Paterson et al., 1983).

[^{3}H] NBMPR is an important tool in NT research as a radiolabelled probe to identify NT inhibitory sites. High affinity binding (<10 nM) of NBMPR to the membrane of erythrocytes (Cass et al., 1974; Jarvis et al., 1982b) and cardiac myocytes (Heaton and Clanachan, 1987) correlates with inhibition of NT and can be inhibited by other NTIs such as dipyridamole and dilazep (Hammond et al., 1981; Hammond and Clanachan 1984; 1985; Williams et al., 1984; Clanachan et al., 1987). In some cultured neoplastic cells, such as Novikoff

hepatoma cells (Plagemann and Wohlhueter, 1985) and Walker 256 carcinosarcoma cells (Paterson et al., 1985), NT systems lack high affinity binding sites for NBMPR, and NT in these cells is not inhibited by NBMPR at concentrations up to 1 μM. This led to the discovery of NBMPR- insensitive facilitated diffusion NT systems. In some types of neoplastic cells, such as cultured mouse leukemia L1210 cells (Belt, 1983) and HeLa cells (Dahlig-Harley et al., 1981), these two subtypes (NBMPR-sensitive and NBMPR-insensitive) of NT systems coexist.

It is obvious that the facilitated NT systems display heterogeneity among different tissues and species. This heterogeneity can be represented as three distinct forms;

(1) sub-types of NT systems have been described based on the sensitivities to NBMPR. Human erythrocytes possess an NBMPR-sensitive facilitated diffusion NT mechanism that is inhibited by low concentrations (< 10 nM) of NBMPR (Paterson et al., 1983; Belt and Noel, 1988; Gati and Paterson, 1989). NBMPR-insensitive transporters, that are only inhibited by higher NBMPR concentrations (>1-5 μM) or are not inhibited by NBMPR, have been reported in several cultured tumor cells (Paterson et al., 1987; Jarvis, 1987) and rat erythrocytes (Jarvis and Young, 1986).

(2) sub-types of NT systems can also be described in terms of sensitivities to dipyridamole. It has long been known that dipyridamole does not potentiate the effects of adenosine in rat heart (Hopkins and Goldie, 1971). This has been demonstrated to be due to the lower affinity of dipyridamole for NT systems in rat tissues by studies of the measurement of dipyridamole-induced inhibition of NBMPR binding and potentiation of adenosine-induced effects (Williams et al., 1984). Also, the single population of NBMPR binding sites in

some CNS membrane preparations, that were inhibited in a biphasic manner by dipyridamole and dilazep (Hammond and Clanachan, 1984; 1985), suggests that two or more subtypes of the transporter exist that can be distinguished by dipyridamole but not by NBMPR.

(3) subtypes of NT systems have also been suggested on the basis of differences in substrate specificity (Thampy and Barnes, 1983a, 1983b).

F. Pharmacology of NTIs

NTIs enhance and prolong the pharmacological effects of adenosine which include CNS sedative effects, cardiovascular effects (such as coronary vasodilation, inhibition of platelet aggregation, prevention of dysrhythmias) and inhibition of lipolysis and neuronal activity.

The pharmacological effects of dilazep and dipyridamole have been the most extensively studied. They are known as coronary vasodilators (Afonso, 1970; Nonaka and Ueno, 1978) and cause significant increases in coronary blood flow. Dilazep and lidoflazine also have calcium entry blocking effects. Most of the NTIs have a vasodilating action which may be due to their potentiation of the vasodilating action of adenosine since it can be neutralized by the adenosine receptor antagonist, theophylline (Sollevi et al., 1984b). The vasodilatory effect of dilazep is believed to be due to both its adenosine potentiating effect (Mustafa, 1979; Tonini et al., 1983) and its calcium entry blocking effect (Mustafa, 1979; Tonini et al., 1983). It has also been shown that dilazep can attenuate coronary occlusion-induced decrease in myocardial pH; therefore, it attenuates myocardial acidosis during ischaemia (Haga et al., 1986). Dipyridamole is also a well-known coronary vasodilator. It causes a substantial increase in coronary blood flow (Feldman et al., 1981) which is

believed to be mediated by an increase in endogenous adenosine concentrations (Homback et al., 1979). It has also been demonstrated to have a beneficial effect on myocardial infarct size by reducing myocardial ischaemic injury (Adolfsson et al., 1982). Dipyridamole has the effect of inhibiting platelet aggregation and is used clinically as an antithrombotic agent (Ritchie and Harker, 1977).

The finding that the existence of the NBMPR-sensitive and -insensitive NT transporters led to evaluations of the combined use of cytotoxic nucleoside analogues (such as tubercidin) and NTIs (such as NBMPR) in cancer chemotherapy. It is hoped that such combinations could specifically kill tumor cells and protect host cells because of their different sensitivities to NBMPR. A great effort has been made in the search for nucleoside analogues with antineoplastic activity (Montgomery, 1974; Townsend and Cheng, 1974) and most of these nucleosides are believed to produce their antineoplastic action by a cytotoxic effect. In contrast to the cellular uptake of adenosine which terminates its action, the effect of cytotoxic nucleosides are only exerted following their uptake into cells (Paterson et al., 1981). Thus the use of potentially lethal doses of cytotoxic nucleosides, in combination with host-protective doses of NTIs may achieve a substantial kill of neoplastic cells and protect the host tissues.

The coadministration of cytotoxic nucleosides with NTIs have also been investigated in some viral (De Clercq and Torrence, 1978; Hahn, 1979) and parasitic (el Kouni, 1991) diseases.

Many nucleoside analogues as well as adenosine itself have been synthesized as potential therapeutic agents (Paterson et al., 1981). Most of the clinical uses of NTIs so far are limited to the cardiovascular system.

Dilazep has a potent vasodilating action *in vivo* and has been shown to

be effective in the chronic treatment of ischaemic heart disease (Giovanni et al., 1986), such as angina pectoris and is without any significant side effects (Takasaki et al., 1977; Giovanni et al., 1986). Dipyridamole has been used clinically in the treatment of angina and in the measurement of coronary sinus blood flow and coronary reserve in patients with hypertension (Pichard et al., 1981), coronary disease (Becker, 1976; Feldman et al., 1981), and valvular disease (Pichard et al., 1983). Dipyridamole is also used in the thallium scanning of patients with angina to demarcate the zone of defective perfusion (Mullane and Williams, 1990).

G. Rationale

The foregoing review has outlined the important aspects concerning the cardiovascular effects of adenosine, and has attempted to indicate the importance of NT mechanisms in the control of extracellular adenosine concentrations. It also indicates that drug-induced modulation of NT activity has significant consequences, not only for the control of the physiological and pharmacological effects adenosine, but also in drug combination therapies of neoplastic, parasitic and viral diseases. The properties of inhibitors of NT systems have been investigated previously in a number of ways, including measurement of their effects on initial rates of nucleoside fluxes, on nucleoside uptake and on the site-specific binding of the NT ligand, [^{3}H]NBMPR. Their ability to potentiate the cardiovascular and other effects of adenosine has been noted, and agents such as dipyridamole and dilazep have been used clinically as coronary vasodilators in patients with ischaemic heart disease. Their potential utility as indirect adenosinemimetics and in drug combination strategies in chemotherapy has stimulated a renewed interest in these agents

that has been further intensified by the discovery of subtypes of NT systems.

Little is known about the pharmacological effects of the newer NT inhibitors. In preliminary clinical studies with NBMPR, a pronounced hypotensive action was observed. This effect is undesirable for most situations, particularly in chemotherapy where hypotension may further aggravate subjective symptoms such as nausea and malaise. It is not clear if NTI-induced hypotension is due to inhibition of NT *per se* leading to a potentiation of the vasodilatory actions of endogenous adenosine or to other mechanisms unrelated to NT inhibition. The experiments described in this thesis explore NTI-adenosine interactions *in vivo* in a quantitative manner which allow comparison of the direct effects of several NTIs on hemodynamics as well as their effects on both the magnitude and duration of the cardiovascular effects of exogenous adenosine. It was hoped that this experimental approach would help determine if drug-induced NT inhibition (measured as potentiation of exogenous adenosine) could be separated from drug-induced hypotension. The specific objectives of this research were to compare several NTIs (dipyridamole, dilazep) and NTI prodrugs (NBMPR-P, NBTGR-P and NBdAdo-P) with respect to

(1) their effects on arterial pressure and heart rate,

(2) their ability to potentiate adenosine-induced changes in arterial pressure and heart rate,

(3) their ability to prolong the duration of action of adenosine on arterial pressure and heart rate, and

(4) their pharmacokinetic profile as judged from time-course experiments that followed NTI-adenosine interactions for up to 60 min following administration.

During the conduct of these experiments, adenosine-induced

tachycardia was observed in conscious rabbits and this response was reversed by NTIs. This unexpected finding was investigated in a further series of experiments where the mechanism for the adenosine-induced tachycardia was studied using various receptor antagonists and the specificity of the NTI-induced reversal of the tachycardia was examined using a second vasodilator, sodium nitroprusside.

II METHODS

Animals

Rabbits (Flemish Giant 3.0 to 4.0 kg) and rats (Sprague-Dawley 250 to 350 g) of either gender were used in these studies. They were obtained from the Health Sciences Laboratory Animal Services, University of Alberta.

Both species were housed under standard conditions of light and temperature. Commercial food and water were supplied *ad libitum*.

Hemodynamic Measurements

The rats were chosen randomly for each experiment and anaesthetized by an intraperitoneal injection of sodium pentobarbital (45 $mg.kg^{-1}$ body weight). Anaesthesia usually ensued in 5 to 15 min and generally lasted about 1 hr. Anaesthesia was maintained by giving additional small amounts of barbiturate (up to 10 $mg.kg^{-1}$ body weight) every hr. The stock solution of sodium pentobarbital (65 $mg.ml^{-1}$) was diluted to 16.25 $mg.ml^{-1}$ to simplify accurate maintenance dose administration.

When the rat was completely anaesthetized, which was judged by an absence of a withdrawal reflex following a gentle foot pinch, a vertical incision was made along the midline of the neck from thyroid cartilage to within 1 cm above the suprasternal notch. The incision was deepened by blunt dissection and the strap muscles were identified. The right carotid artery was identified between the trachea and the strap muscles. It was separated from the attached jugular vein and nerves (vagal and superior cervical sympathetic trunks), tied at the cephalic end and clamped at the caudal end. Between these two ends and close to the cephalic end, a hole was cut in the artery through which a polythene (Quik-Cath, 20G) catheter, that was filled with heparinised saline,

was inserted. A ligature was then loosely tied around the artery and the inserted cannula. Following removal of the clamp, the cannula was pushed further (1 cm) along the carotid artery and securely tied in position. The arterial line was then connected to a blood pressure transducer. A second cannula was introduced into the right external jugular vein in a similar manner for drug administration and connected to a 3-way tap.

Conscious rabbits were selected randomly for each study. The rabbit was kept in a box in which it could move its head freely during the experimental period. A special dark and quiet experimental room was used in order to decrease the stress on the animals. The rabbit was kept warm by keeping the room temperature constant at 25°C. Its ears were shaved and xylene was rubbed on their surface to distend the vessels to facilitate their visualisation. An arterial catheter (Quik-Cath, 20G), previously filled with heparinised saline, was inserted into the central ear artery and connected to a blood pressure transducer. A second catheter (Winged Infusion Set, 21G), that was filled with saline, was inserted into the marginal vein on the contralateral ear and connected to a 3-way tap, through which drug solutions could be administered.

A model 7D polygraph (Grass Instrument Company, 101 Old Colony Avenue, Quincy, MA, 02169, U.S.A.) was used in these studies to record arterial blood pressure (BP) and heart rate (HR). It was calibrated before each experiment. A calibrated amplitude of BP was set up by a sphygmomanometer. HR was measured via a tachagraph (Grass Model 7P4) triggerred from the arterial pressure pulse. Chart recordings were made at a paper speed of 10 $mm.min^{-1}$.

Drug Administration

Bolus injections of drug solutions were given to rats and rabbits in volumes of 1 $ml.kg^{-1}$ body weight for rat and 0.2 $ml.kg^{-1}$ body weight for rabbit. Saline (0.5 ml) was infused through same catheter within 1 s after each drug injection to ensure complete drug administration.

Adenosine dose-response curves were prepared by giving graded doses of adenosine, 20 $\mu g.kg^{-1}$ (75 $nmole.kg^{-1}$), 50 $\mu g.kg^{-1}$ (187 $nmole.kg^{-1}$), 100 $\mu g.kg^{-1}$ (374 $nmole.kg^{-1}$), 200 $\mu g.kg^{-1}$ (748 $nmole.kg^{-1}$), 500 $\mu g.kg^{-1}$ (1870 $nmole.kg^{-1}$) by IV bolus. The time interval between doses was 5 min by which time response to adenosine had completely recovered.

Dose-response curves of the actions of the various NTIs were obtained by giving graded, cumulative doses of NTIs every 10 min, while at 5 min after each dose of NTI, adenosine (374 $nmole.kg^{-1}$) was administered to determine the degree of adenosine potentiation.

An examination of the **time-course** of the actions of the various NTIs was performed by giving a single dose of each NTI followed by doses of adenosine (374 $nmole.kg^{-1}$) every 10 min, beginning 5 min, and up to 55 min, after NTI administration.

Dose-response curves of adenosine and time-courses of the effects of each NTI were prepared in a similar manner in rats and rabbits.

In rabbits, the effects of adenosine were compared to a second vasodilator, sodium nitroprusside (SNP), before and after the NTI, NBMPR-P. The dose ranges of adenosine (75 $nmole.kg^{-1}$, 187 $nmole.kg^{-1}$, 374 $nmole.kg^{-1}$, 748 $nmole.kg^{-1}$, 1870 $nmole.kg^{-1}$) and SNP 0.02 $\mu g.kg^{-1}$ (0.08 $nmole.kg^{-1}$), 0.05 $\mu g.kg^{-1}$ (0.19 $nmole.kg^{-1}$), 0.1 $\mu g.kg^{-1}$ (0.38 $nmole.kg^{-1}$), 0.2 $\mu g.kg^{-1}$ (0.76 $nmole.kg^{-1}$), 0.5 $\mu g.kg^{-1}$ (1.9 $nmole.kg^{-1}$) were chosen to elicit similar degrees of hypotension.

Further experiments to study the effects of receptor antagonists on adenosine- and SNP-induced responses in rabbits were performed by giving selected agonists, adenosine, SNP, histamine and isopropyl-noradrenaline (INA) before and after the ß- adrenoceptor antagonist, propranolol, the histamine H_1 receptor antagonist, diphenhydramine, the histamine H_2 receptor antagonist, cimetidine, and the adenosine A_1 and A_2 receptor antagonist, 8-sulfophenyltheophylline (8-SPT). The doses used are as follows: histamine, 10 $\mu g.kg^{-1}$ (0.033 $\mu mole.kg^{-1}$), INA, 0.03 $\mu g.kg^{-1}$ (0.0001 $\mu mole.kg^{-1}$), diphenhydramine, 1 $mg.kg^{-1}$ (3.4 $\mu mole.kg^{-1}$), cimetidine, 5 $mg.kg^{-1}$ (19.8 $\mu mole.kg^{-1}$), propranolol, 5 $mg.kg^{-1}$, (19.3 $\mu mole.kg^{-1}$), 8-SPT, 10 $mg.kg^{-1}$, (29 $\mu mole.kg^{-1}$). These doses were chosen to elicit similar degrees of hypotension.

Data Analysis

The amplitude of changes in BP and HR were measured from chart recordings that were calibrated before each experiment (200 mmHg was set as full scale (5 cm) deflection). Half time ($t_{1/2}$) recoveries of adenosine-induced BP or HR changes were defined as the time between adenosine injections and the time by which BP or HR responses had recovered by 50%. ED_{15} was defined as the dose required to reduce MAP by 15 mmHg. The two fold prolongation was the dose required to cause a two fold prolongation of adenosine-induced hypotension.

All data in this thesis are expressed as mean ± standard error of the mean (SEM.). Significance levels for the differences between groups were estimated by using analysis of variance (ANOVA) for repeated measures. Individual differences were further analysed by Student's paired t test with Bonferoni's correction for multiple comparisons. Differences were judged to be significant when $P<0.05$.

Drugs and Solutions

Nitrobenzyldeoxyadenosine-5'-phosphate (NBdAdo-P; MW: 526), nitrobenzylthioinosine-5'-phosphate (NBMPR-P; MW:561) and nitrobenzylthioguanosine-5'-phosphate (NBTGR-P; MW:577) were obtained from Dr. Paterson (Department of Pharmacology, University of Alberta). Dilazep (MW:607.7) and dipyridamole (MW: 504.6) were purchased from Sigma Chemical Company, P.O.Box 14508, St. Louis, MO 63178, U.S.A.

Adenosine (MW:267.3), propranolol, isopropylnoradrenaline (INA), histamine, diphenhydramine, cimetidine, 8-sulfophenyltheophylline (8-SPT) were purchased from Sigma Chemical Company.

All drugs were dissolved in 0.9% NaCl (saline) except for dipyridamole that was dissolved in 0.01N HCl-saline.

Heparinised saline contained heparin 50 $iu.ml^{-1}$.

III RESULTS

A. Hemodynamic measurements in anaesthetized rats

Following anaesthesia and instrumentation, baseline values for systolic, diastolic and mean arterial pressure were 124 ± 2 mmHg, 92 ± 2 mmHg and 103 ± 2 mmHg, respectively; heart rate was 382 ± 5 beats.min^{-1}, n=60.

Baseline hemodynamic parameters remained relatively stable throughout the experimental period (2 to 3 hr).

Adenosine, when administered by rapid intravenous bolus in the dose range of 20 to 500 μg.kg^{-1} (75 to 1870 nmole.kg^{-1}), reduced systolic and diastolic arterial pressure in a dose-dependent manner. Similar alterations were observed in both systolic and diastolic pressures; accordingly throughout this thesis, only changes in mean arterial pressure (MAP) are reported.

Adenosine-induced hypotension was accompanied by a dose-dependent bradycardia (Figs. 1A & 1C). Maximal effective doses of adenosine were not investigated in order to avoid severe cardiovascular depression that would have impaired the viability of the experimental preparation. As an index of the duration of action of adenosine, $t_{1/2}$ recovery times for adenosine responses were estimated. Under control conditions, adenosine responses were rapid (less than 10 s at 100 μg.kg^{-1}); $t_{1/2}$ recovery times for BP and HR were similar with the lower doses of adenosine (≤ 374 nmole.kg^{-1}). At the highest dose (1870 nmole.kg^{-1}), the $t_{1/2}$ recovery time for MAP (78 s) was significantly greater than for HR (18 s) (Figs. 1B & 1D).

From these preliminary experiments, a dose of adenosine of 100 μg.kg^{-1} (374 nmole.kg^{-1}) was chosen as a standard, sub-maximal stimulus for the assessment of the actions of NT inhibitors on adenosine responsiveness. Control responses to this dose of adenosine in the various treatment groups are

presented in Table 1 (page 25).

Nucleoside Transport Inhibitors

1. Dipyridamole, 1 to 50 mg.kg^{-1} (2 to 99 μmole.kg^{-1}), reduced MAP (P<0.05) in a dose-dependent manner (Fig. 2A); HR was not significantly affected (Fig. 2D). It also potentiated the duration (Fig. 2C) of adenosine-induced hypotension (P<0.01) and the duration (Fig. 2F) of adenosine-induced bradycardia (P<0.001). The magnitude of adenosine-induced hypotension and bradycardia was not significantly affected (Figs. 2B & 2E). Near maximal increases in adenosine duration of action were attained with doses of dipyridamole (10 to 20 μmole.kg^{-1}) that had no significant effects on MAP and HR *per se*.

A time-course of dipyridamole actions was constructed following a dose of 20 mg.kg^{-1} (40 μmole.kg^{-1}). MAP and HR were not significantly affected although an immediate, but transient, fall in MAP and HR were usually observed following dipyridamole administration (Figs. 3A & 3D).

Dipyridamole potentiated the duration of adenosine-induced hypotension. This potentiation was observed at 5 min (P<0.01) and still evident up to 45 min (P<0.05) following dipyridamole administration (Fig. 3C) while there was no significant potentiation of the magnitude of adenosine-induced hypotension (Fig. 3B). Likewise, dipyridamole also potentiated the duration of adenosine-induced bradycardia although it did not potentiate the magnitude of adenosine-induced bradycardia. The potentiation of the duration of action of adenosine on HR was greatest 5 to 15 min (P<0.01) and was still demonstrable at 55 min (P<0.05) following dipyridamole administration (Fig.

Table 1: Control responses to the standard dose of adenosine (374 $\mu mole.kg^{-1}$) in the various treatment groups of anaesthetized rats.

	MAP		HR	
Treatment Group (n)	Magnitude (mmHg)	Recovery $t_{1/2}$ (s)	Magnitude (beats.min^{-1})	Recovery $t_{1/2}$ (s)
Dipyridamole (n=14)	-26 ± 2	12 ± 1	-66 ± 5	12 ± 1
Dilazep (n=12)	-23 ± 2	11 ± 1	-60 ± 3	12 ± 1
NBMPR-P (n=12)	-26 ± 1	11 ± 1	-57 ± 5	10 ± 1
NBTGR-P (n=12)	-27 ± 2	12 ± 1	-62 ± 7	11 ± 1
NBdAdo-P (n=12)	-24 ± 1	12 ± 1	-64 ± 5	11 ± 1

Responses to adenosine are expressed as the magnitude of the maximal change in mean arterial pressure (MAP) and heart rate (HR); $t_{1/2}$ recovery is the time required for recovery of the responses by 50%. Results are expressed as mean ± SEM for n animals.

3F).

2. Dilazep, 0.1 to 10 mg.kg^{-1} (0.3 to 17 μmole.kg^{-1}), dramatically reduced MAP (P<0.01) from the dose of 0.5 mg.kg^{-1} (0.8 μmole.kg^{-1}) and HR (P<0.05) from the dose of 1 mg.kg^{-1} (1.6 μmole.kg^{-1}) in a dose-dependent manner (Figs. 4A & 4D). It significantly potentiated the duration of adenosine-induced hypotension from the dose of 1 mg.kg^{-1} (P<0.05). It had no significant effects on the magnitude of adenosine-induced hypotension (Fig. 4B) and on the magnitude and duration of adenosine-induced bradycardia effects (Fig. 4E & 4F). A time-course of dilazep effects was constructed with a dose of 5 mg.kg^{-1} (8.3 μmole.kg^{-1}). It caused an immediate and pronounced fall in MAP (P<0.001) and HR (P<0.05). MAP had recovered by 5 min; HR recovered more gradually and had returned to control by 55 min after dilazep administration (Figs. 5A & 5D).

Dilazep potentiated the duration of adenosine-induced hypotension (Fig. 5C) from 5 min (P<0.01) and lasted at least until 55 min (P<0.05). The magnitude of adenosine-induced hypotension was not significantly affected (Fig. 5B). Dilazep also significantly (P<0.05) potentiated the duration of adenosine-induced bradycardia at 5 min after its administration (Fig. 5F) although it antagonized (P<0.05) the bradycardic effect of adenosine (Fig. 5E).

3. NBMPR-P, 1 to 50 mg.kg^{-1} (1.8 to 89 μmole.kg^{-1}), caused a dose-dependent decrease in MAP (P<0.05) (Fig. 6A). HR was not significantly affected (Fig. 6D). It potentiated the duration of adenosine-induced hypotension (P<0.01) at doses ≥ 2 mg.kg^{-1} (≥ 3.6μmole.kg^{-1}). NBMPR-P did not significantly potentiate the magnitude (Fig. 6B) of adenosine-induced hypotension or the

magnitude (Fig. 6E) and duration (Fig. 6F) of adenosine-induced bradycardia.

A time-course of NBMPR-P effects was constructed following a dose of 20 $mg.kg^{-1}$ (36 $\mu mole.kg^{-1}$). Immediate falls in MAP and HR were observed after drug administration. These effects recovered within 5 min (Figs. 7A & 7D).

NBMPR-P, at the dose used, did not potentiate the magnitude (Fig. 7B) or duration (Fig. 7C) of adenosine-induced hypotension or the magnitude (Fig. 7E) of adenosine-induced bradycardia. The duration (Fig. 7F) of adenosine-induced bradycardia was significantly potentiated 15 min after administration, an effect that lasted at least until 55 min after NBMPR-P administration.

4. NBTGR-P, 1 to 50 $mg.kg^{-1}$ (1.7 to 87 $\mu mole.kg^{-1}$), reduced MAP in a dose-dependent manner (Fig. 8A) ($P< 0.01$) but had no significant effect on HR even at the largest dose (Fig. 8D). It significantly potentiated the duration (Fig. 8C) of adenosine-induced hypotension at doses ≥ 2 $mg.kg^{-1}$ ($P<0.001$). NBTGR-P did not significantly potentiate the magnitude of adenosine-induced hypotension or the magnitude of adenosine-induced bradycardia (Figs. 8B & 8E), but significantly ($P<0.01$) potentiated the duration of adenosine-induced bradycardia in a dose-dependent manner (Fig. 8F).

A time-course of NBTGR-P effects was constructed following the dose of 20 $mg.kg^{-1}$ (35 $\mu mole.kg^{-1}$). MAP and HR were not significantly affected after drug administration (Figs. 9A & 9D).

The duration (Fig. 9C) of adenosine-induced hypotension and the duration (Fig. 9F) of adenosine-induced bradycardia were significantly ($P<0.05$) potentiated (Figs. 9C & 9F). The magnitude of adenosine-induced hypotension (Fig. 9B) and bradycardia (Fig. 9E) was not significantly affected by NBTGR-P.

5. NBdAdo-P, 1 to 50 mg.kg^{-1} (1.9 to 95 μmole.kg^{-1}), had no significant effects on MAP (Fig. 10A) or HR (Fig. 10D). It significantly potentiated the magnitude (Fig. 10B) (P<0.001) and duration (Fig. 10C) (P<0.01) of adenosine-induced hypotension and had its greatest effect at a dose of 9.5 μmole.kg^{-1}. Its effects on the magnitude (Fig. 10E) and duration (Fig. 10F) of adenosine-induced bradycardia were not very pronounced.

A time-course of NBdAdo-P effects was constructed after a dose of 20 mg.kg^{-1} (38 μmole.kg^{-1}). No significant falls in MAP and HR were observed after drug administration (Figs. 11A & 11D).

Potentiation of the duration of adenosine-induced hypotension by NBdAdo-P was significant (P<0.05) and long lasting (Fig. 11C). It attained its greatest effect at 5 min and potentiation remained at this level for up to 55 min. It had no significant potentiating effect on the magnitude of adenosine-induced hypotension or on the magnitude and duration of adenosine-induced bradycardia (Figs. 11B & 11E & 11F).

B. Hemodynamic measurements in conscious rabbits

After instrumentation and stabilization, baseline values for systolic, diastolic and MAP were 91 ± 3 mmHg, 58 ± 2 mmHg and 69 ± 2 mmHg, respectively; HR was 215 ± 6 beats.min^{-1}, n=28. Baseline hemodynamic parameters remained relatively stable throughout the experimental period of 1 to 2 hr.

Adenosine, when administered by rapid intravenous bolus in the dose range of 20 to 500 μg.kg^{-1} (75 to 1870 nmole.kg^{-1}), reduced systolic and diastolic arterial pressure in a dose-dependent manner. Similar alterations were

observed in both systolic and diastolic pressures. In contrast to the response in anaesthetized rats, adenosine-induced hypotension was accompanied by a dose-dependent tachycardia (Figs. 12A & 12C) rather than a bradycardia. Recovery $t_{1/2}$ times for arterial pressures and HR (Figs. 12B & 12D) were similar at each of the dose ranges.

From these preliminary experiments, a dose of adenosine of 100 $\mu g.kg^{-1}$ (374 $nmole.kg^{-1}$) was chosen as a standard, submaximal stimulus for the assessment of the actions of NT inhibitors on adenosine responsiveness.

Nucleoside Transport Inhibitors

1. Dipyridamole, 1 $mg.kg^{-1}$ (2 $\mu mole.kg^{-1}$) did not significantly affect MAP and HR (Figs. 13A & 13D).

Dipyridamole significantly potentiated the duration of adenosine-induced hypotension 5 min ($P<0.01$) after administration and this potentiation had recovered by 25 min (Figs. 13C). It had no significant influence on the magnitude of adenosine-induced hypotension (Fig. 13B). The effect of dipyridamole on adenosine-induced tachycardia was more complicated. Adenosine-induced tachycardia was reversed to bradycardia ($P<0.001$) 5 min after dipyridamole administration and began to recover by 35 min (Fig. 13E). The potentiating effect of dipyridamole on the $t_{1/2}$ recovery time of adenosine-induced HR effects was significant at 5 min ($P<0.05$) and recovered gradually thereafter (Fig. 13F).

2. Dilazep, 0.5 $mg.kg^{-1}$ (0.8 $\mu mole.kg^{-1}$), decreased MAP ($P<0.05$) 5 min after its administration and this effect lasted until 45 min (Fig. 14A). The HR was

not significantly affected (Fig. 14D). Dilazep caused a marked potentiation ($P<0.001$) of the duration of adenosine-induced hypotension and this effect was maintained during the experimental period (Fig. 14C). It had no significant influence on the magnitude of adenosine-induced hypotension (Fig. 14B). It significantly ($P<0.001$) reversed adenosine-induced tachycardia to bradycardia, and this effect was longer lasting than that caused by dipyridamole (Fig. 14E). A biphasic effect of adenosine on HR was observed at 45 and 55 min after dilazep administration (not shown in fig). The potentiating effect of dilazep on the duration of adenosine-induced changes in HR were significant ($P<0.05$) at 5 min and maintained during the experimental period (Fig. 14F).

3. NBMPR-P, 1 $mg.kg^{-1}$ (1.8 $\mu mole.kg^{-1}$), decreased MAP ($P<0.05$) at 5 and 10 min after drug administration, an effect that gradually recovered thereafter (Fig. 15A). It did not significantly affect HR (Fig. 15D).

NBMPR-P potentiated both the magnitude ($P<0.01$) and the duration ($P<0.01$) of adenosine-induced hypotension. The potentiation of the magnitude was observed between 35 and 55 min and the potentiation of the duration between 5 and 55 min after NBMPR-P administration (Figs. 15B & 15C). Adenosine-induced tachycardia was reversed to a bradycardia after NBMPR-P administration ($P<0.05$) and this effect was also long lasting (Fig. 15E). Again, a biphasic effect of adenosine on HR (consisting of an initial bradycardia followed by a tachycardia) was usually observed between 25 min and 55 min after drug administration. The prolongation of adenosine-induced HR effects was significant at 5 min ($P<0.05$) and lasted throughout the experimental period (Fig. 15F).

4. NBTGR-P, 1 $mg.kg^{-1}$ (1.7 $\mu mole.kg^{-1}$), decreased MAP significantly

($P<0.05$) 15 min after its administration (Fig. 16A) and significantly increased HR between 5 and 45 min after administration. The maximal effects on HR were at 5 min ($P<0.001$) and were still significant at 45 min ($P<0.05$) although that was a tendency for the effect to recover gradually to control levels (Fig. 16D).

The potentiating effect of NBTGR-P on the duration of adenosine-induced hypotension was maximal at 5 min ($P<0.001$) after drug administration and recovered gradually (Fig. 16C). The magnitude of adenosine-induced hypotension was not significantly potentiated (Fig. 16B). Adenosine-induced tachycardia was reversed to bradycardia ($P<0.001$) 5 min following NBTGR-P administration, and recovered to tachycardia at 35 min ($P<0.01$) although this tachycardia was less than that caused by adenosine in the absence of NBTGR-P (Fig. 16E). The potentiating effect of NBTGR-P on the duration of adenosine-induced HR effects was significant ($P<0.05$) at 5 min and 35 min (Fig. 16F).

5. NBdAdo-P, 1 $mg.kg^{-1}$ (1.9 $\mu mole.kg^{-1}$), caused a decrease in MAP ($P<0.05$) at 15 min (Fig. 17A) and a marked increase in HR ($P<0.01$) between 5 and 25 min after its administration (Fig. 17D).

NBdAdo-P potentiated the magnitude of adenosine-induced hypotension at 25 min ($P<0.05$) and lasted at least until 55 min ($P<0.05$) after its administration (Fig. 17B). The potentiating effect of NBdAdo-P on the duration of adenosine-induced hypotension was maximal at 5 min ($P<0.001$) and recovered gradually during the experimental period (Fig. 17C). As for all the other NTIs, the tachycardia effect of adenosine was reversed to bradycardia at 5 min ($P<0.001$) and recovered to bradycardia at 35 min after NBdAdo-P administration (Fig. 17E). As for all the other NTIs, during the recovery time, a biphasic effect was usually observed between 15 and 25 min that consisted of a

bradycardia followed by a tachycardia (not shown in fig.). NBdAdo-P significantly potentiated the duration of adenosine-induced HR effects 5 min ($P<0.001$) and was still effective 55 min ($P<0.05$) after its administration (Fig. 17F).

C. Comparison of adenosine and SNP responses in absence and presence of NBMPR-P.

Adenosine dose-response curves (at the doses of 20, 50, 100, 200, 500 $\mu g.kg^{-1}$), and sodium nitroprusside (SNP) dose-response curves (at the doses of 0.02, 0.05, 0.1, 0.2, 0.5 $\mu g.kg^{-1}$) at which it caused the similar degree of falls in MAP as those caused by adenosine, were determined in normal conscious rabbits before and after NBMPR-P administration.

In untreated animals, both adenosine and SNP produced dose-dependent reductions in MAP that were accompanied by increases in HR (Figs. 18A & 18C). When HR changes were plotted against drug-induced changes in MAP (Fig. 19), tachycardia was less with equi-effective hypotensive doses of adenosine than with SNP. Following administration of NBMPR-P (1 $mg.kg^{-1}$), adenosine and SNP still elicited dose-dependent falls in MAP (Figs. 18B & 18D) that were similar to those observed in the absence of NBMPR-P. In the presence of NBMPR-P, SNP-induced hypotension was accompanied by increases in HR, although HR increases were less ($P< 0.05$) (Figs. 18D & 19). Although adenosine caused falls in MAP following NBMPR-P administration that were similar to those for SNP, HR did not increase and adenosine-induced bradycardia was observed (Figs. 18B & 19). Consequently, NBMPR-P caused a reversal of adenosine-induced changes in HR.

D. Effect of receptor antagonists on adenosine-induced arterial pressure and heart rate responses.

The effects of several different receptor antagonists on adenosine-induced arterial pressure and HR responses were examined in normal conscious rabbits (Figs. 20 & 21).

Similar degrees of hypotension and tachycardia were elicited by each of the following agonists: adenosine (100 $\mu g.kg^{-1}$), SNP (10 $\mu g.kg^{-1}$), histamine (10 $\mu g.kg^{-1}$) and INA (0.03 $\mu g.kg^{-1}$). These doses produced falls in MAP of 20 ± 1, 13 ± 3, 10 ± 1 and 13 ± 1 mmHg, respectively, and increases in HR of 74 ± 4, 53 ± 4, 43 ± 4 and 70 ± 4 $beats.min^{-1}$, respectively. Responses in the presence of the various antagonists are expressed as a percentage of these control values.

Following the administration of the histamine H_1 receptor antagonist, diphenhydramine (1 $mg.kg^{-1}$) and H_2 receptor antagonist, cimetidine (5 $mg.kg^{-1}$), the MAP and HR effects of histamine were significantly attenuated but the effects of adenosine were not significantly altered. After the administration of the ß-adrenoceptor antagonist, propranolol (5 $mg.kg^{-1}$), which significantly (*P<0.001*) antagonised the hypotension and tachycardia of INA, adenosine-induced hypotension ($P<0.05$) was only slightly inhibited and adenosine-induced tachycardia was not significantly affected. After the administration of the adenosine A_1 and A_2 receptor antagonist, 8-sulfophenyltheophylline (8-SPT, 10 $mg.kg^{-1}$), both the MAP and HR effects of adenosine were significantly inhibited ($P<0.05$).

Figure 1: Arterial pressure (A, B) and heart rate (C, D) responses to adenosine in anaesthetized rats. Maximal changes in the magnitude of mean arterial pressure (MAP, mmHg, ordinate, A) and heart rate (HR, beats.min^{-1}, ordinate, C) following rapid intravenous administration of different doses of adenosine (abscissae) are shown. As an index of the duration of action of adenosine , $t_{1/2}$ recovery times (s) for MAP (ordinate, B) and HR (ordinate, D) responses are presented. Data points represent the mean ± SEM of 10 different experiments.

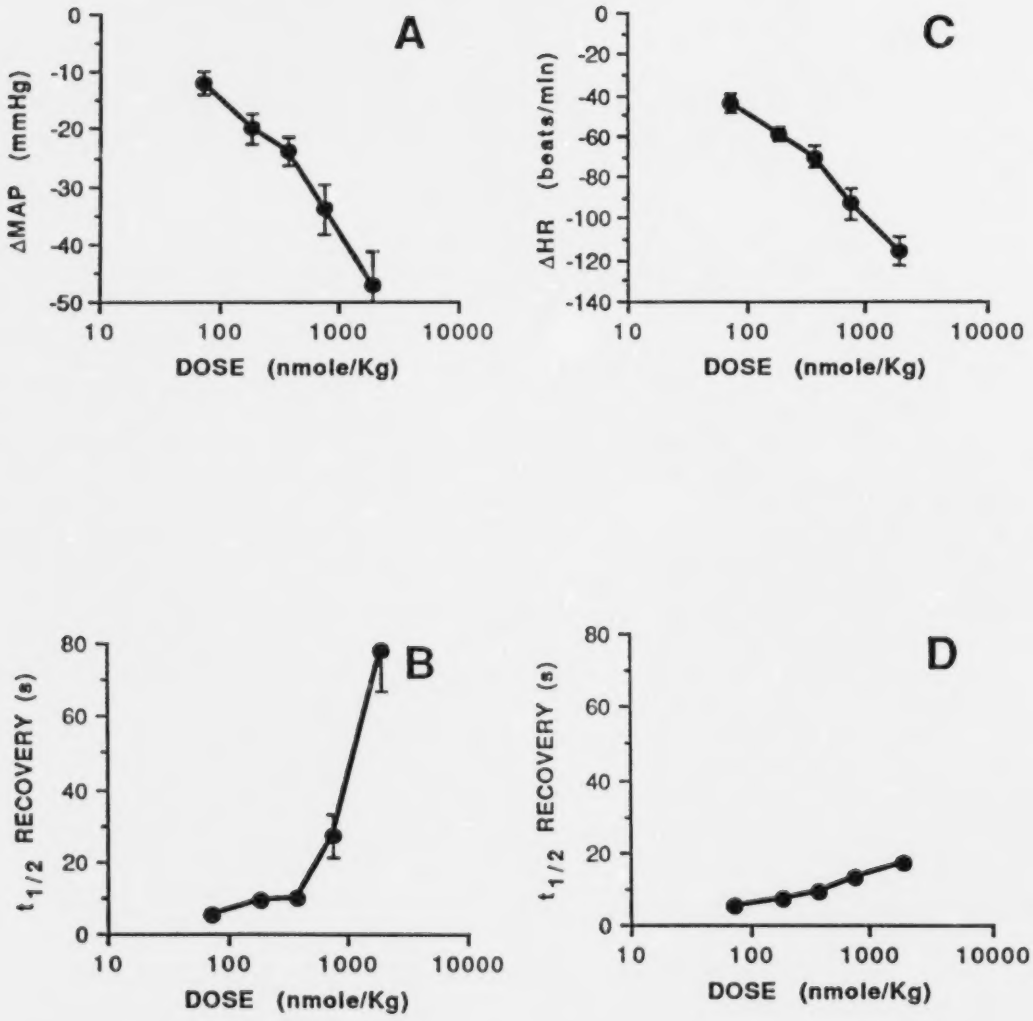
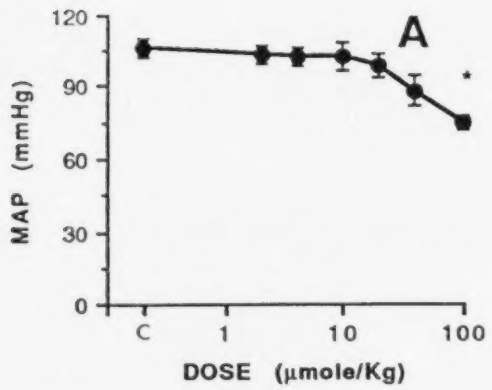
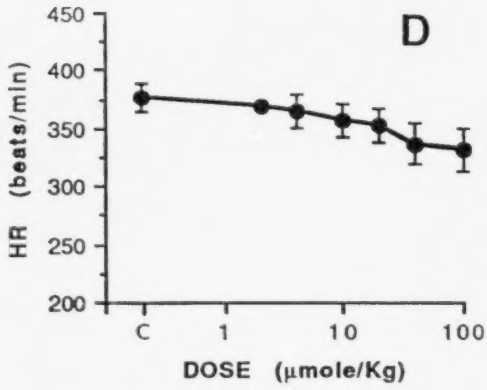
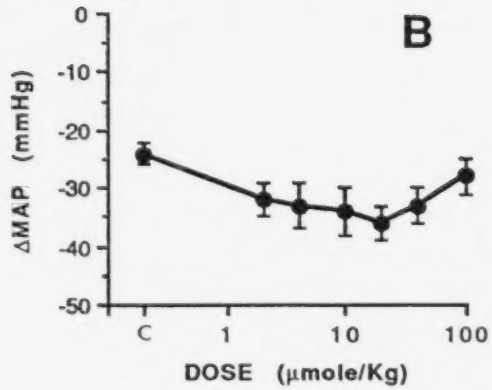
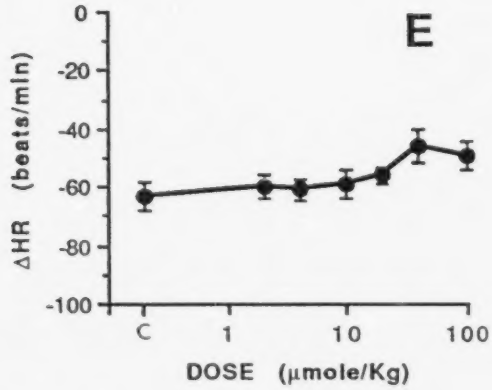

A
ΔMAP (mmHg)
0
-10
-20
-30
-40
-50
10
100
1000
10000
DOSE (nmole/Kg)
C
ΔHR (beats/min)
0
-20
-40
-60
-80
-100
-120
-140
10
100
1000
10000
DOSE (nmole/Kg)
B
t1/2 RECOVERY (s)
80
60
40
20
0
10
100
1000
10000
DOSE (nmole/Kg)
D
t1/2 RECOVERY (s)
80
60
40
20
0
10
100
1000
10000
DOSE (nmole/Kg)

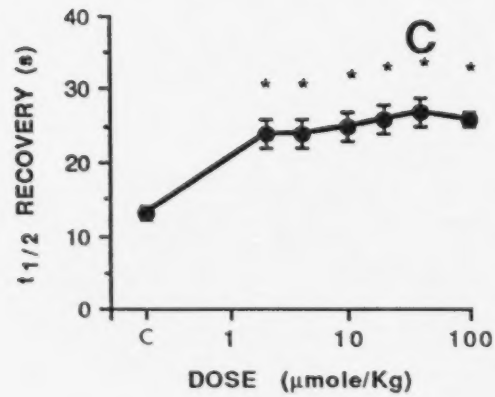
Figure 2: Dipyridamole dose-response relationships in anaesthetized rats. Maximal changes in the magnitude of the mean arterial pressure (MAP, mmHg, ordinate, A) and heart rate (HR, beats.min^{-1}, ordinate, D) following intravenous administration of different doses of dipyridamole (abscissae) are shown. Also changes in the magnitude of the MAP (B) and HR (E) responses and the $t_{1/2}$ recovery times (s) of the MAP (C) and HR (F) responses to adenosine given 5 min after each dose of dipyridamole are presented. Data points represent the mean ± SEM of 6 different experiments. Asterisks indicate points that are significantly different ($P< 0.05$) from pre-drug baseline values.


A
MAP (mmHg)
120
90
60
30
0
C
1
10
100
DOSE (µmole/Kg)


D
HR (beats/min)
450
400
350
300
250
200
C
1
10
100
DOSE (µmole/Kg)


B
ΔMAP (mmHg)
0
-10
-20
-30
-40
-50
C
1
10
100
DOSE (µmole/Kg)


E
ΔHR (beats/min)
0
-20
-40
-60
-80
-100
C
1
10
100
DOSE (µmole/Kg)


C
t1/2 RECOVERY (s)
40
30
20
10
0
C
1
10
100
DOSE (µmole/Kg)

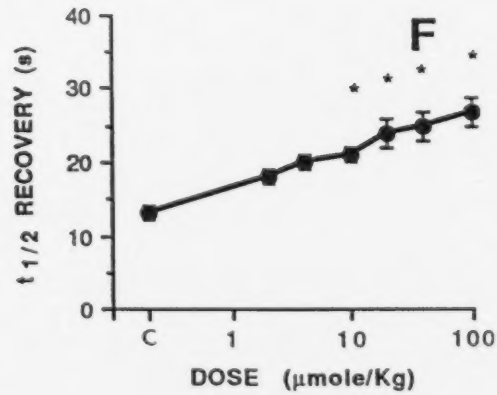
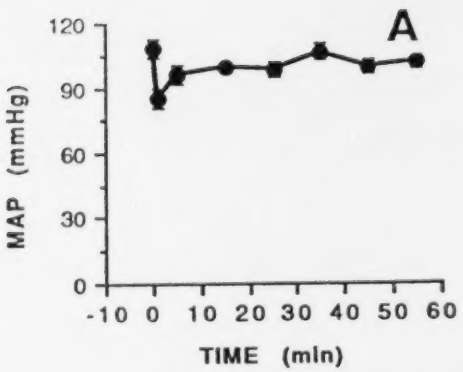
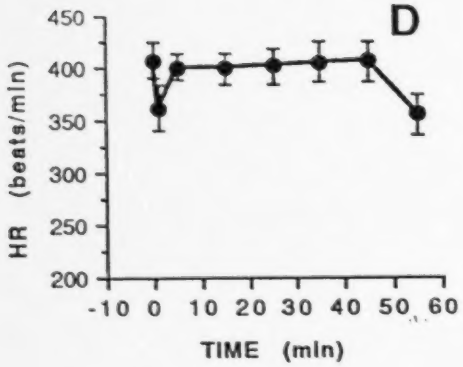
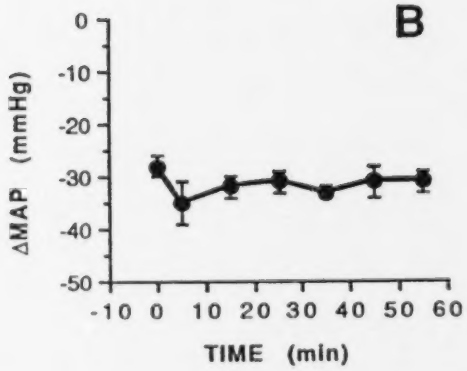
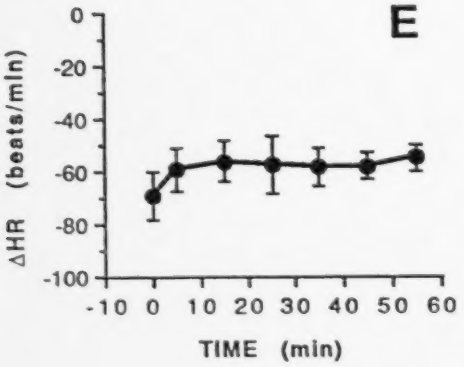

F
t1/2 RECOVERY (s)
40
30
20
10
0
C
1
10
100
DOSE (µmole/Kg)

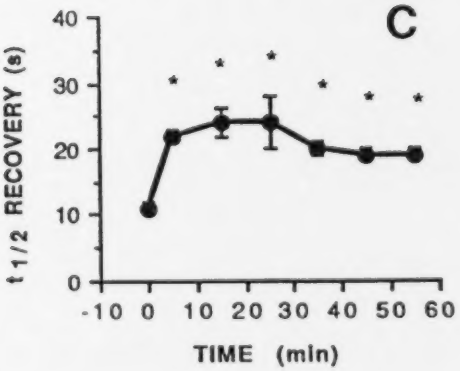
Figure 3: Dipyridamole (50 mg.kg^{-1}) time-course in anaesthetized rats. The changes in the magnitude of the mean arterial pressure (MAP, mmHg, ordinate, A) and heart rate (HR, beats.min^{-1}, ordinate, D) with time (min, abscissae) following intravenous administration of a single dose of dipyridamole are shown. Also changes in the magnitude of the MAP (B) and HR (E) responses and the $t_{1/2}$ recovery times (s) of the MAP (C) and HR (F) responses to adenosine at various times following dipyridamole administration are presented. Data points represent the mean ± SEM of 6 different experiments. Asterisks indicate points that are significantly different ($P< 0.05$) from pre-drug baseline values.


A
MAP (mmHg)
120
90
60
30
0
-10 0 10 20 30 40 50 60
TIME (min)


D
HR (beats/min)
450
400
350
300
250
200
-10 0 10 20 30 40 50 60
TIME (min)


B
ΔMAP (mmHg)
0
-10
-20
-30
-40
-50
-10 0 10 20 30 40 50 60
TIME (min)


E
ΔHR (beats/min)
0
-20
-40
-60
-80
-100
-10 0 10 20 30 40 50 60
TIME (min)


C
t 1/2 RECOVERY (s)
40
30
20
10
0
-10 0 10 20 30 40 50 60
TIME (min)

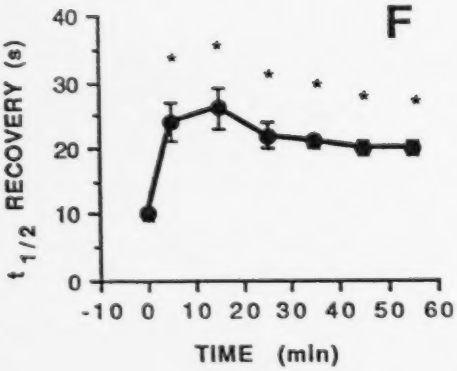
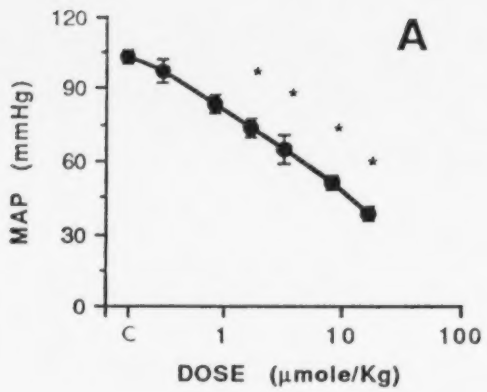
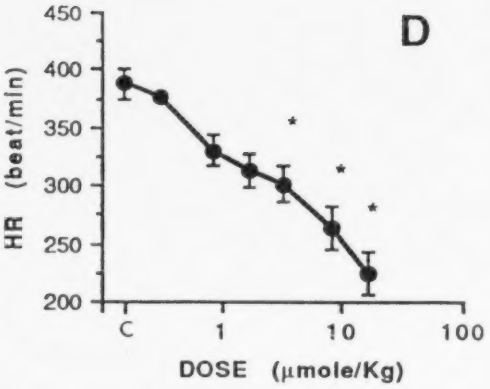
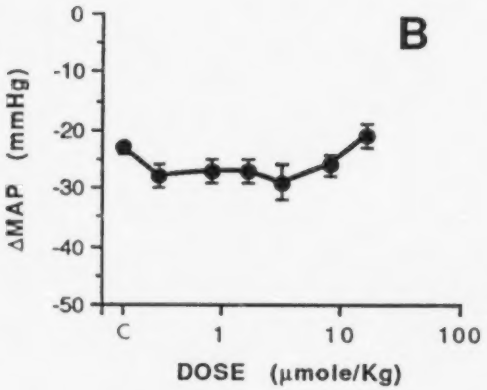
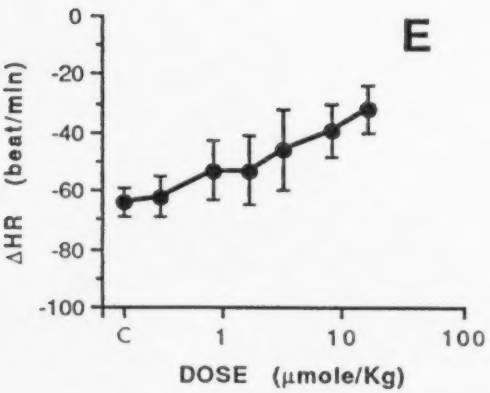

F
t 1/2 RECOVERY (s)
40
30
20
10
0
-10 0 10 20 30 40 50 60
TIME (min)

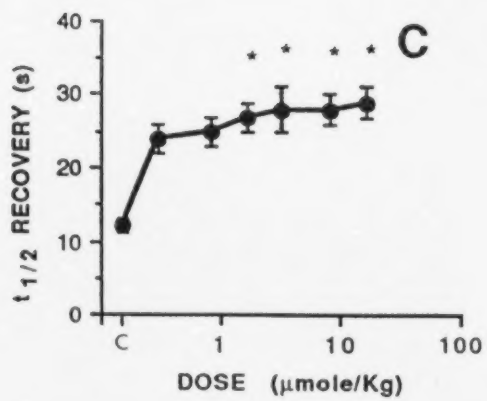
Figure 4: Dilazep dose-response relationships in anaesthetized rats. Maximal changes in the magnitude of the mean arterial pressure (MAP, mmHg, ordinate, A) and heart rate (HR, beats.min^{-1}, ordinate, D) following intravenous administration of different doses of dilazep (abscissae) are shown. Also changes in the magnitude of the MAP (B) and HR (E) responses and the $t_{1/2}$ recovery times (s) of the MAP (C) and HR (F) responses to adenosine given 5 min after each dose of dilazep are presented. Data points represent the mean ± SEM of 6 different experiments. Asterisks indicate points that are significantly different ($P< 0.05$) from pre-drug baseline values.


A
MAP (mmHg)
120
90
60
30
0
C
1
10
100
DOSE (µmole/Kg)


D
HR (beat/min)
450
400
350
300
250
200
C
1
10
100
DOSE (µmole/Kg)


B
ΔMAP (mmHg)
0
-10
-20
-30
-40
-50
C
1
10
100
DOSE (µmole/Kg)


E
ΔHR (beat/min)
0
-20
-40
-60
-80
-100
C
1
10
100
DOSE (µmole/Kg)


C
t 1/2 RECOVERY (s)
40
30
20
10
0
C
1
10
100
DOSE (µmole/Kg)

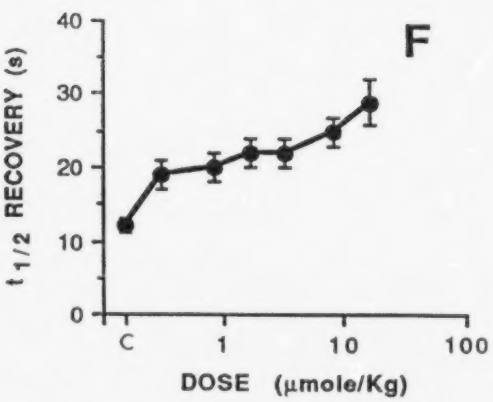
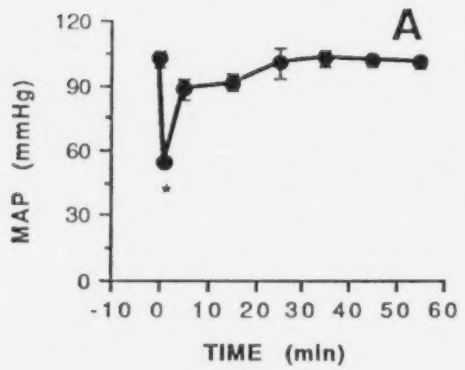
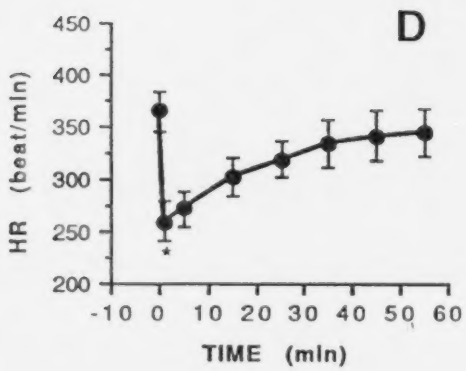
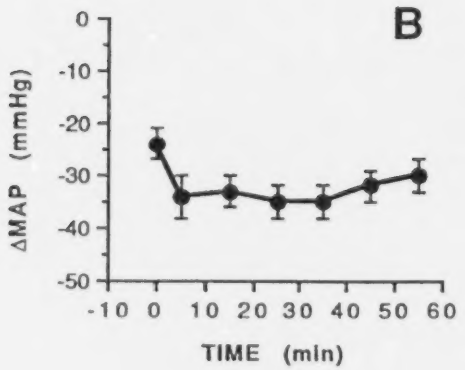
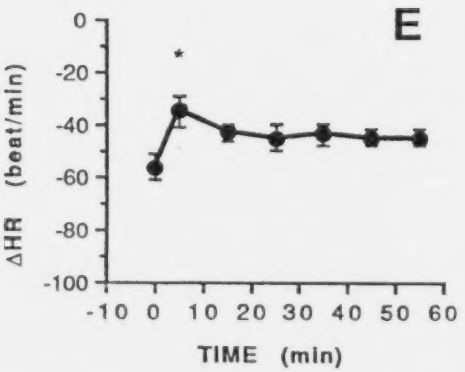

F
t 1/2 RECOVERY (s)
40
30
20
10
0
C
1
10
100
DOSE (µmole/Kg)

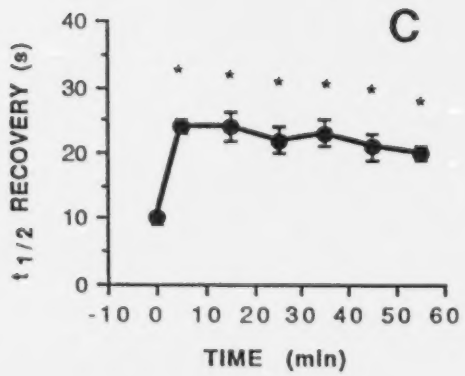
Figure 5: Dilazep (10mg.kg^{-1}) time-course in anaesthetized rats. The changes in the magnitude of the mean arterial pressure (MAP, mmHg, ordinate, A) and heart rate (HR, beats.min^{-1}, ordinate, D) with time (min, abscissae) following intravenous administration of a single dose of dilazep are shown. Also changes in the magnitude of the MAP (B) and HR (E) responses and the $t_{1/2}$ recovery times (s) of the MAP (C) and HR (F) responses to adenosine at various times following dilazep administration are presented. Data points represent the mean ± SEM of 6 different experiments. Asterisks indicate points that are significantly different ($P< 0.05$) from pre-drug baseline values.


A
MAP (mmHg)
120
90
60
30
0
-10 0 10 20 30 40 50 60
TIME (min)


D
HR (beat/min)
450
400
350
300
250
200
-10 0 10 20 30 40 50 60
TIME (min)


B
ΔMAP (mmHg)
0
-10
-20
-30
-40
-50
-10 0 10 20 30 40 50 60
TIME (min)


E
ΔHR (beat/min)
0
-20
-40
-60
-80
-100
-10 0 10 20 30 40 50 60
TIME (min)


C
t 1/2 RECOVERY (s)
40
30
20
10
0
-10 0 10 20 30 40 50 60
TIME (min)

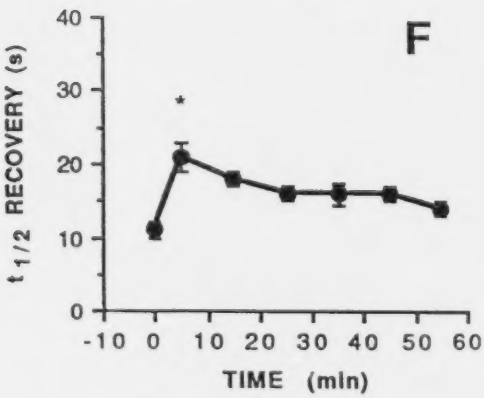

F
t 1/2 RECOVERY (s)
40
30
20
10
0
-10 0 10 20 30 40 50 60
TIME (min)

Figure 6: NBMPR-P dose-response relationships in anaesthetized rats. Maximal changes in the magnitude of the mean arterial pressure (MAP, mmHg, ordinate, A) and heart rate (HR, beats.min^{-1}, ordinate, D) following intravenous administration of different doses of NBMPR-P (abscissae) are shown. Also changes in the magnitude of the MAP (B) and HR (E) responses and the $t_{1/2}$ recovery times (s) of the MAP (C) and HR (F) responses to adenosine given 5 min after each dose of NBMPR-P are presented. Data points represent the mean ± SEM of 6 different experiments. Asterisks indicate points that are significantly different ($P< 0.05$) from pre-drug baseline values.

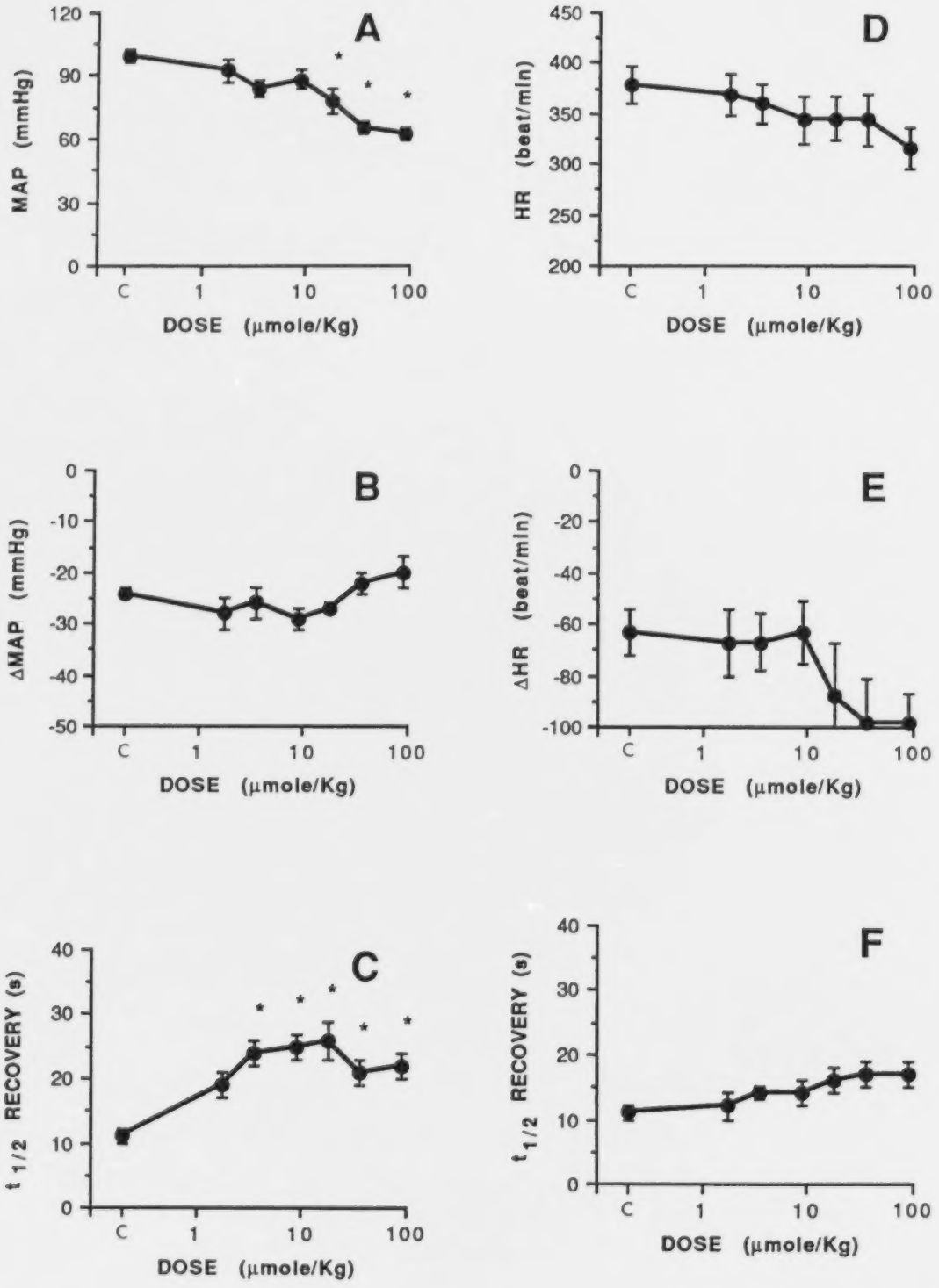
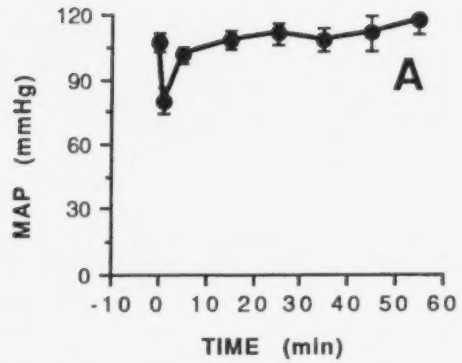
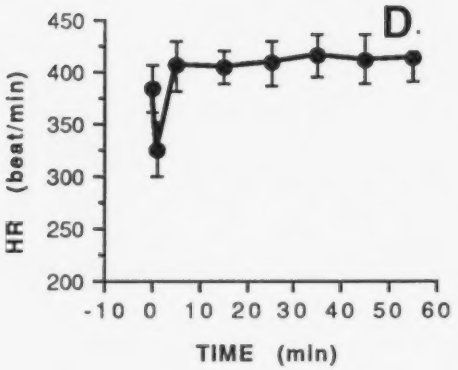
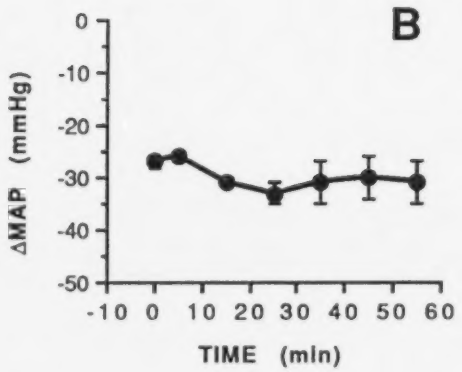
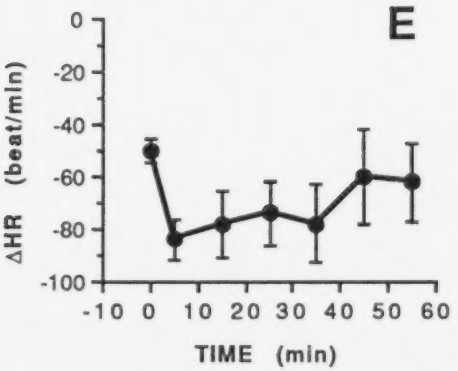

A
MAP (mmHg)
120
90
60
30
0
C
1
10
100
DOSE (µmole/Kg)
D
HR (beat/min)
450
400
350
300
250
200
C
1
10
100
DOSE (µmole/Kg)
B
ΔMAP (mmHg)
0
-10
-20
-30
-40
-50
C
1
10
100
DOSE (µmole/Kg)
E
ΔHR (beat/min)
0
-20
-40
-60
-80
-100
C
1
10
100
DOSE (µmole/Kg)
C
t 1/2 RECOVERY (s)
40
30
20
10
0
C
1
10
100
DOSE (µmole/Kg)
F
t1/2 RECOVERY (s)
40
30
20
10
0
C
1
10
100
DOSE (µmole/Kg)

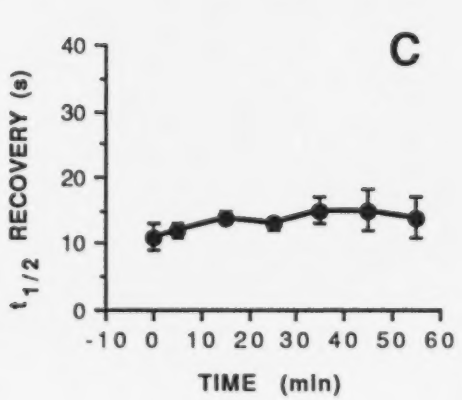
Figure 7: NBMPR-P (20 $mg.kg^{-1}$) time-course in anaesthetized rats. The changes in the magnitude of the mean arterial pressure (MAP, mmHg, ordinate, A) and heart rate (HR, $beats.min^{-1}$, ordinate, D) with time (min, abscissae) following intravenous administration of a single dose of NBMPR-P are shown. Also changes in the magnitude of the MAP (B) and HR (E) responses and the $t_{1/2}$ recovery times (s) of the MAP (C) and HR (F) responses to adenosine at various times following NBMPR-P administration are presented. Data points represent the mean ± SEM of 5 different experiments. Asterisks indicate points that are significantly different
($P< 0.05$) from pre-drug baseline values.


A
MAP (mmHg)
120
90
60
30
0
-10 0 10 20 30 40 50 60
TIME (min)


D.
HR (beat/min)
450
400
350
300
250
200
-10 0 10 20 30 40 50 60
TIME (min)


B
ΔMAP (mmHg)
0
-10
-20
-30
-40
-50
-10 0 10 20 30 40 50 60
TIME (min)


E
ΔHR (beat/min)
0
-20
-40
-60
-80
-100
-10 0 10 20 30 40 50 60
TIME (min)


C
t1/2 RECOVERY (s)
40
30
20
10
0
-10 0 10 20 30 40 50 60
TIME (min)

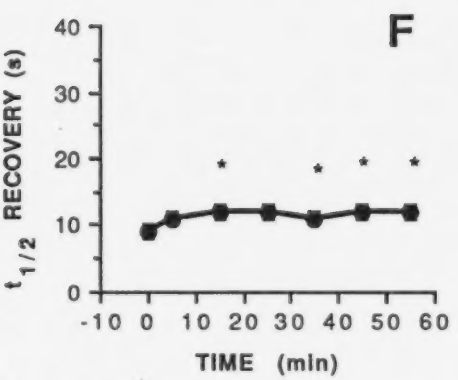
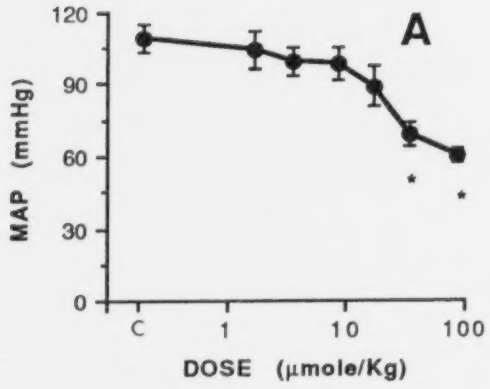
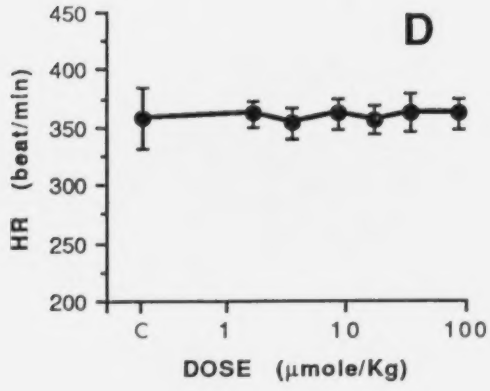
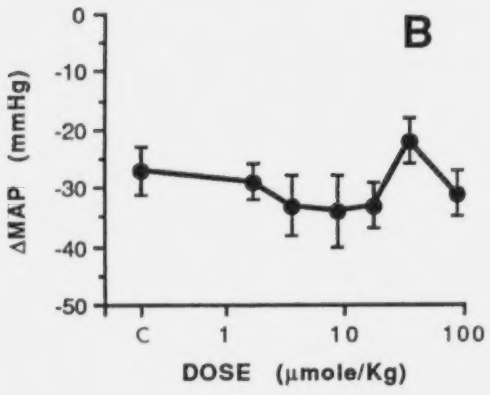
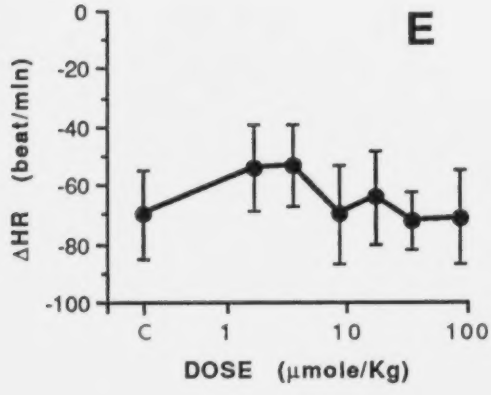

F
t1/2 RECOVERY (s)
40
30
20
10
0
-10 0 10 20 30 40 50 60
TIME (min)

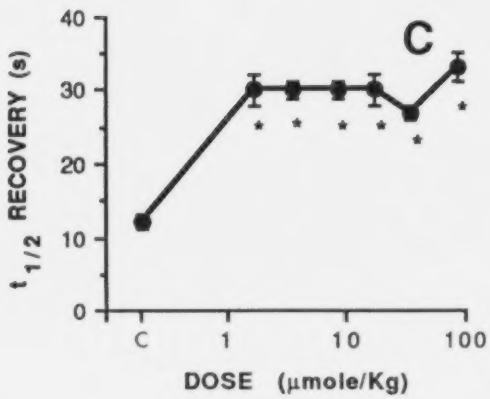
Figure 8: NBTGR-P dose-response relationships in anaesthetized rats. Maximal changes in the magnitude of the mean arterial pressure (MAP, mmHg, ordinate, A) and heart rate (HR, beats.min^{-1}, ordinate, D) following intravenous administration of different doses of NBTGR-P (abscissae) are shown. Also changes in the magnitude of the MAP (B) and HR (E) responses and the $t_{1/2}$ recovery times (s) of the MAP (C) and HR (F) responses to adenosine given 5 min after each dose of NBTGR-P are presented. Data points represent the mean ± SEM of 5 different experiments. Asterisks indicate points that are significantly different ($P< 0.05$) from pre-drug baseline values.


A
MAP (mmHg)
120
90
60
30
0
C
1
10
100
DOSE (µmole/Kg)


D
HR (beat/min)
450
400
350
300
250
200
C
1
10
100
DOSE (µmole/Kg)


B
ΔMAP (mmHg)
0
-10
-20
-30
-40
-50
C
1
10
100
DOSE (µmole/Kg)


E
ΔHR (beat/min)
0
-20
-40
-60
-80
-100
C
1
10
100
DOSE (µmole/Kg)


C
t 1/2 RECOVERY (s)
40
30
20
10
0
C
1
10
100
DOSE (µmole/Kg)

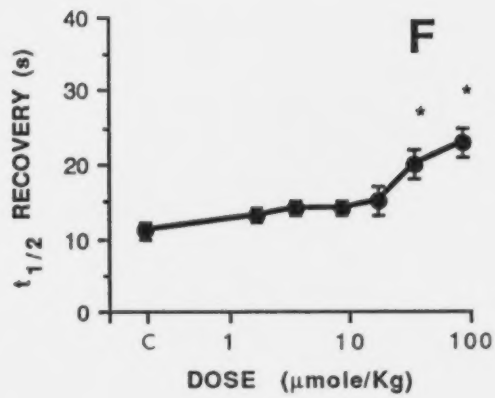
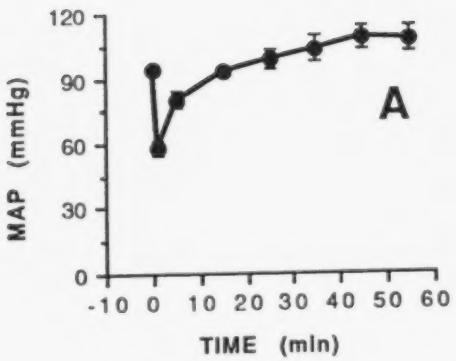
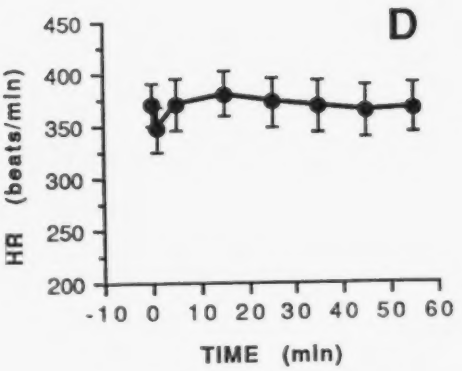
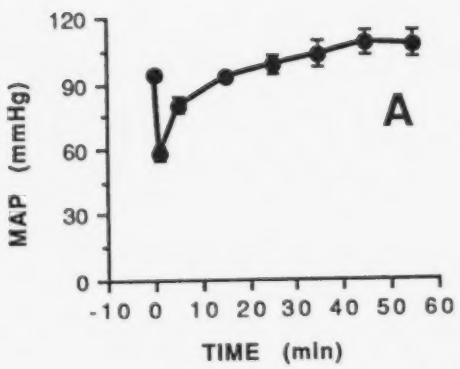
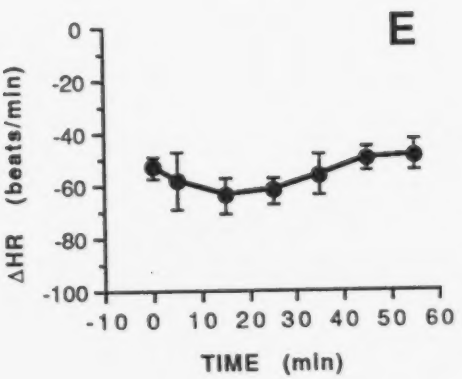

F
t 1/2 RECOVERY (s)
40
30
20
10
0
C
1
10
100
DOSE (µmole/Kg)

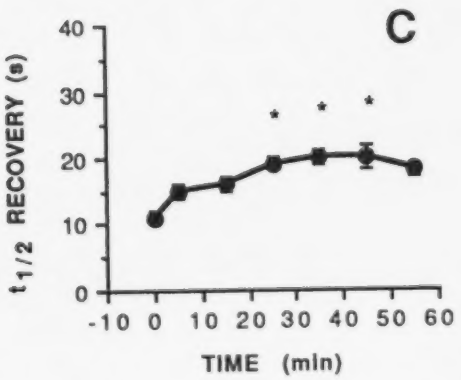
Figure 9: NBTGR-P (20 $mg.kg^{-1}$) time-course in anaesthetized rats. The changes in the magnitude of the mean arterial pressure (MAP, mmHg, ordinate, A) and heart rate (HR, $beats.min^{-1}$, ordinate, D) with time (min, abscissae) following intravenous administration of a single dose of NBTGR-P are shown. Also changes in the magnitude of the MAP (B) and HR (E) responses and the $t_{1/2}$ recovery times (s) of the MAP (C) and HR (F) responses to adenosine at various times following NBTGR-P administration are presented. Data points represent the mean ± SEM of 6 different experiments. Asterisks indicate points that are significantly different
($P< 0.05$) from pre-drug baseline values.


A
MAP (mmHg)
120
90
60
30
0
-10 0 10 20 30 40 50 60
TIME (min)


D
HR (beats/min)
450
400
350
300
250
200
-10 0 10 20 30 40 50 60
TIME (min)


A
MAP (mmHg)
120
90
60
30
0
-10 0 10 20 30 40 50 60
TIME (min)


E
ΔHR (beats/min)
0
-20
-40
-60
-80
-100
-10 0 10 20 30 40 50 60
TIME (min)


C
t1/2 RECOVERY (s)
40
30
20
10
0
-10 0 10 20 30 40 50 60
TIME (min)

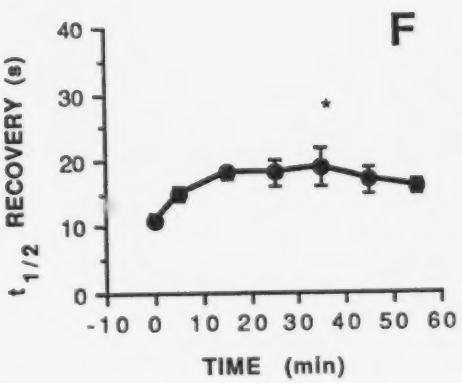

F
t1/2 RECOVERY (s)
40
30
20
10
0
-10 0 10 20 30 40 50 60
TIME (min)

Figure 10: NBdAdo-P dose-response relationships in anaesthetized rats. Maximal changes in the magnitude of the mean arterial pressure (MAP, mmHg, ordinate, A) and heart rate (HR, beats.min^{-1}, ordinate, D) following intravenous administration of different doses of NBdAdo-P (abscissae) are shown. Also changes in the magnitude of the MAP (B) and HR (E) responses and the $t_{1/2}$ recovery times (s) of the MAP (C) and HR (F) responses to adenosine given 5 min after each dose of NBdAdo-P are presented. Data points represent the mean ± SEM of 6 different experiments. Asterisks indicate points that are significantly different
($P< 0.05$) from pre-drug baseline values.

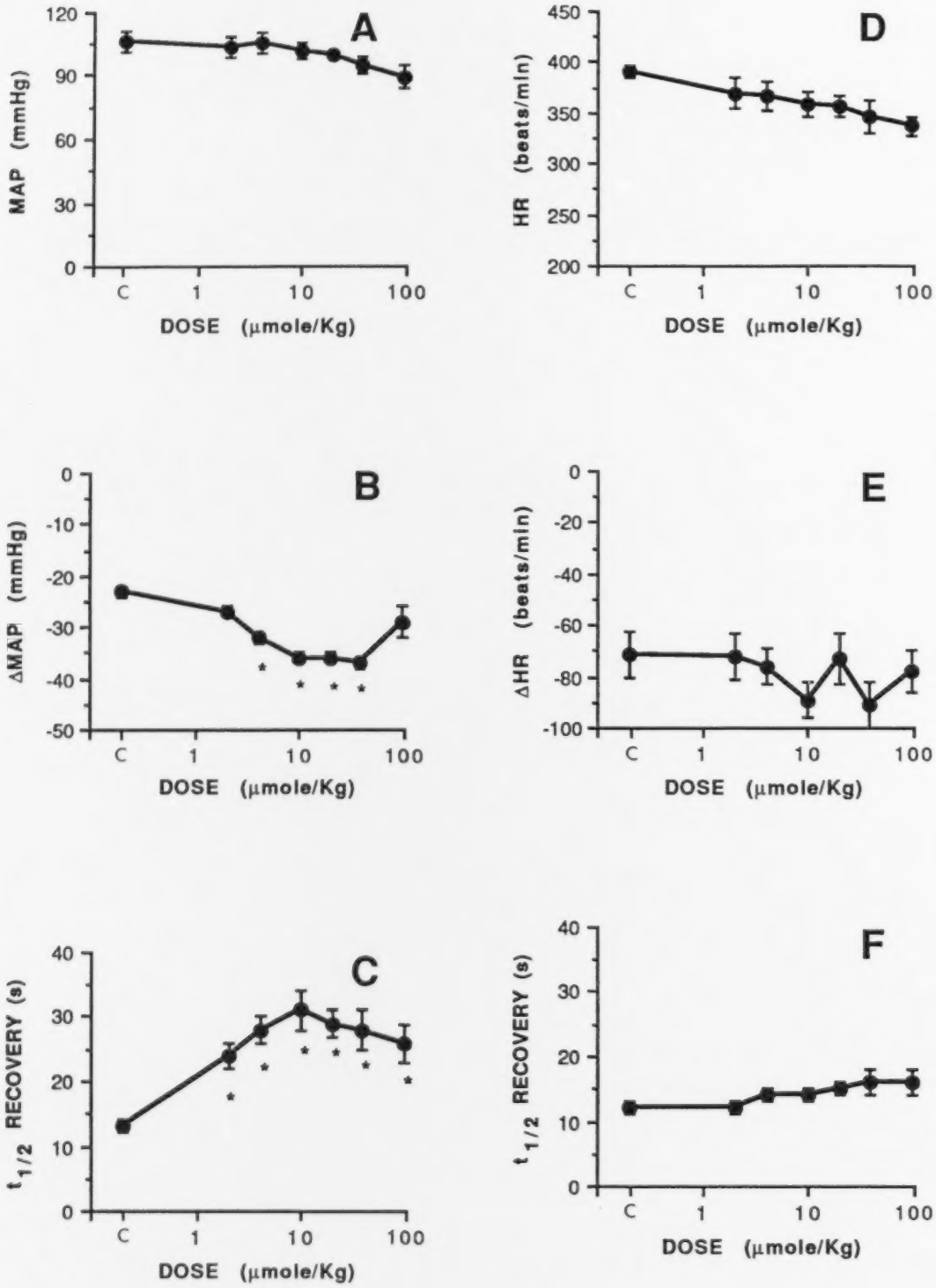
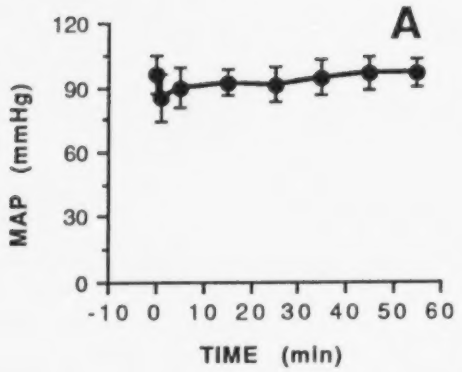
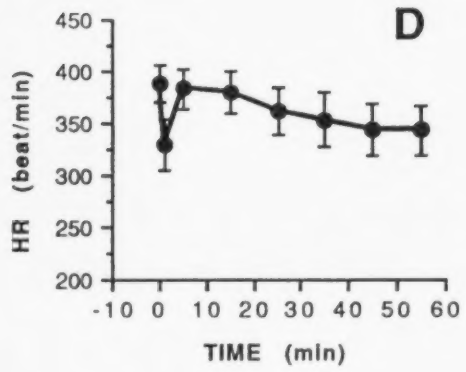
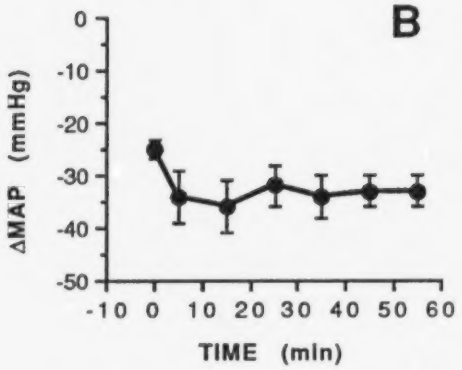
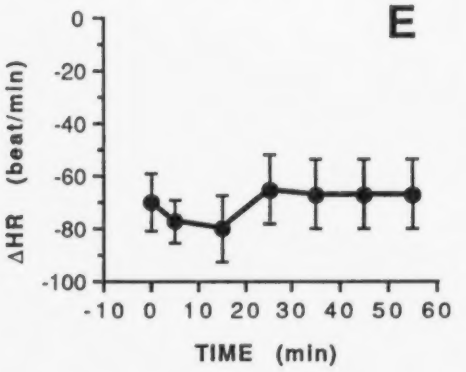

A
MAP (mmHg)
120
90
60
30
0
C
1
10
100
DOSE (µmole/Kg)
D
HR (beats/min)
450
400
350
300
250
200
C
1
10
100
DOSE (µmole/Kg)
B
ΔMAP (mmHg)
0
-10
-20
-30
-40
-50
C
1
10
100
DOSE (µmole/Kg)
E
ΔHR (beats/min)
0
-20
-40
-60
-80
-100
C
1
10
100
DOSE (µmole/Kg)
C
t1/2 RECOVERY (s)
40
30
20
10
0
C
1
10
100
DOSE (µmole/Kg)
F
t1/2 RECOVERY (s)
40
30
20
10
0
C
1
10
100
DOSE (µmole/Kg)

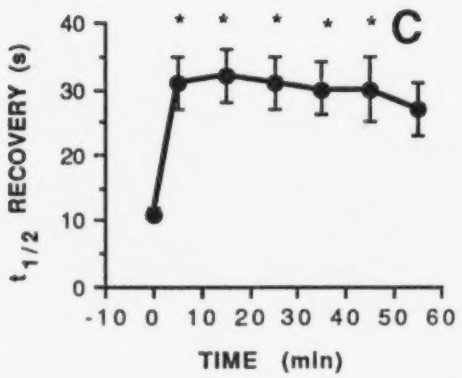
Figure 11: NBdAdo-P (20 $mg.kg^{-1}$) time-course in anaesthetized rats. The changes in the magnitude of the mean arterial pressure (MAP, mmHg, ordinate, A) and heart rate (HR, $beats.min^{-1}$, ordinate, D) with time (min, abscissae) following intravenous administration of a single dose of NBdAdo-P are shown. Also changes in the magnitude of the MAP (B) and HR (E) responses and the $t_{1/2}$ recovery times (s) of the MAP (C) and HR (F) responses to adenosine at various times following NBdAdo-P administration are presented. Data points represent the mean ± SEM of 6 different experiments. Asterisks indicate points that are significantly different
($P< 0.05$) from pre-drug baseline values.


A
MAP (mmHg)
120
90
60
30
0
-10 0 10 20 30 40 50 60
TIME (min)


D
HR (beat/min)
450
400
350
300
250
200
-10 0 10 20 30 40 50 60
TIME (min)


B
ΔMAP (mmHg)
0
-10
-20
-30
-40
-50
-10 0 10 20 30 40 50 60
TIME (min)


E
ΔHR (beat/min)
0
-20
-40
-60
-80
-100
-10 0 10 20 30 40 50 60
TIME (min)


C
t 1/2 RECOVERY (s)
40
30
20
10
0
-10 0 10 20 30 40 50 60
TIME (min)

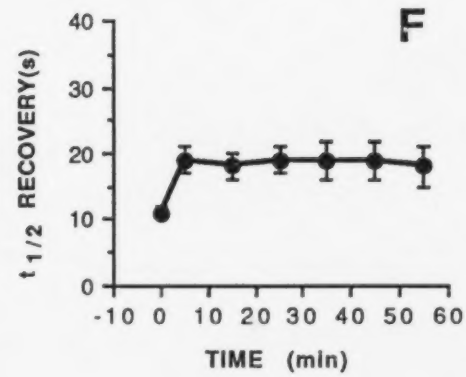

F
t 1/2 RECOVERY(s)
40
30
20
10
0
-10 0 10 20 30 40 50 60
TIME (min)

Figure 12: Arterial pressure (A, B) and heart rate (C, D) responses to adenosine in conscious rabbits. Maximal changes in the magnitude of the mean arterial pressure (MAP, mmHg, ordinate, A) and heart rate (HR, beats.min^{-1}, ordinate, C) following rapid intravenous administration of different doses of adenosine (abscissae) are shown. As an index of the duration of action of adenosine, $t_{1/2}$ recovery times (s) for MAP (ordinate, B) and HR (ordinate, D) responses are presented. Data points represent the mean ± SEM of 10 different experiments.

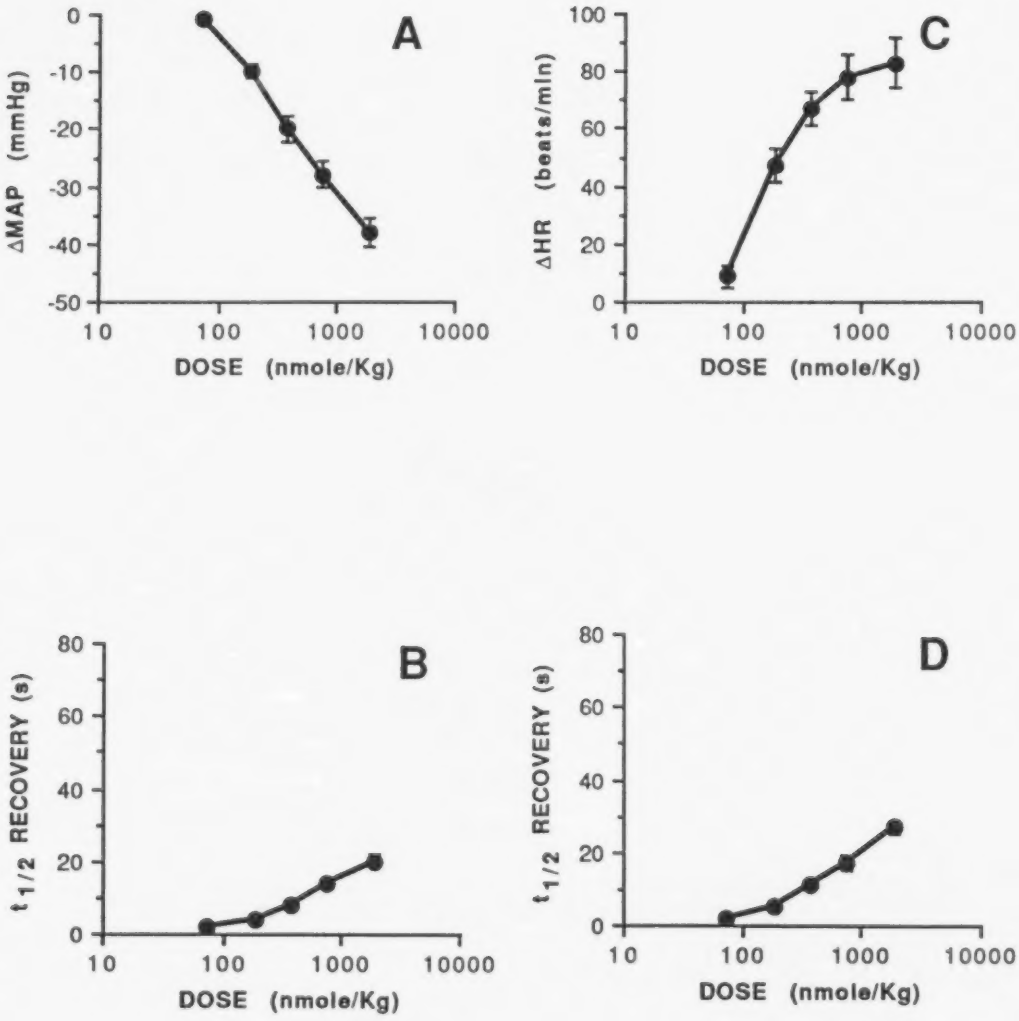
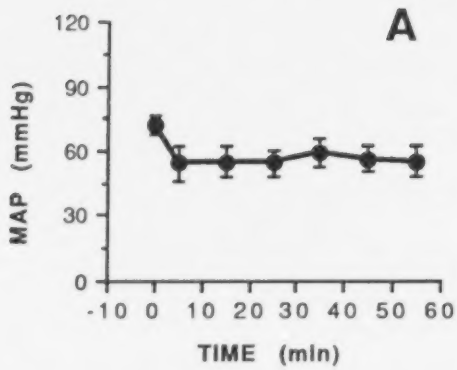
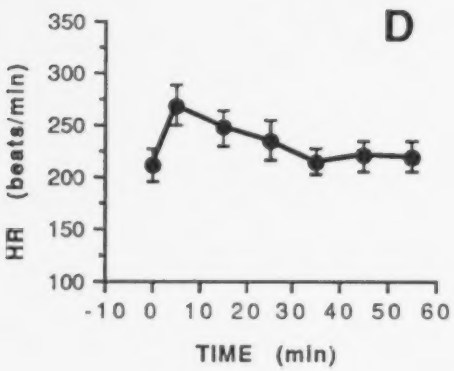
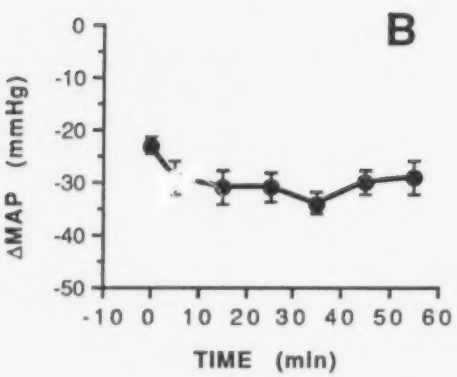
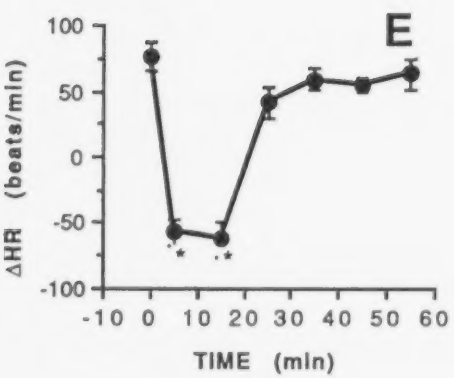

A
ΔMAP (mmHg)
0
-10
-20
-30
-40
-50
10
100
1000
10000
DOSE (nmole/Kg)
C
ΔHR (beats/min)
100
80
60
40
20
0
10
100
1000
10000
DOSE (nmole/Kg)
B
t 1/2 RECOVERY (s)
80
60
40
20
0
10
100
1000
10000
DOSE (nmole/Kg)
D
t 1/2 RECOVERY (s)
80
60
40
20
0
10
100
1000
10000
DOSE (nmole/Kg)

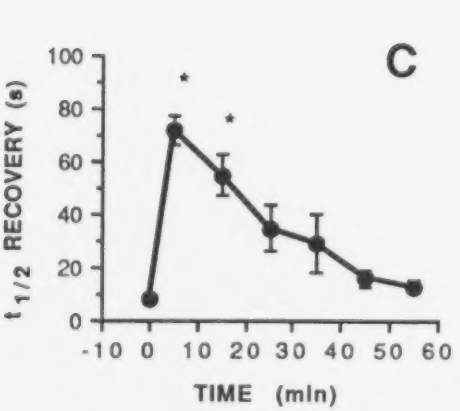
Figure 13: Dipyridamole 1 $mg.kg^{-1}$ (2 $\mu mole.kg^{-1}$) time-course in conscious rabbits. The changes in the magnitude of the mean arterial pressure (MAP, mmHg, ordinate, A) and heart rate (HR, $beats.min^{-1}$, ordinate, D) with time (min, abscissae) following intravenous administration of a single dose of dipyridamole are shown. Also, changes in the magnitude of MAP (B) and HR (E) responses and the $t_{1/2}$ recovery times (s) of the MAP (C) and HR (F) responses to adenosine at various times following dipyridamole administration are presented. Data points represent the mean ± SEM of 5 different experiments. Asterisks indicate points that are significantly different ($P< 0.05$) from pre-drug baseline values.


A
MAP (mmHg)
120
90
60
30
0
-10 0 10 20 30 40 50 60
TIME (min)


D
HR (beats/min)
350
300
250
200
150
100
-10 0 10 20 30 40 50 60
TIME (min)


B
ΔMAP (mmHg)
0
-10
-20
-30
-40
-50
-10 0 10 20 30 40 50 60
TIME (min)


E
ΔHR (beats/min)
100
50
0
-50
-100
-10 0 10 20 30 40 50 60
TIME (min)


C
t1/2 RECOVERY (s)
100
80
60
40
20
0
-10 0 10 20 30 40 50 60
TIME (min)

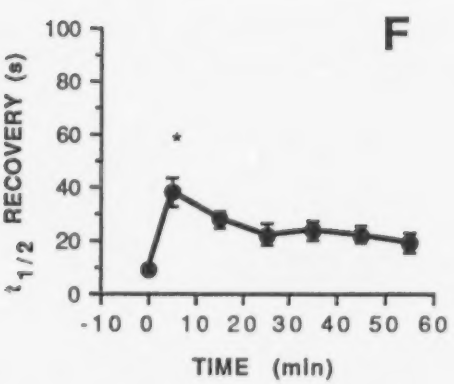
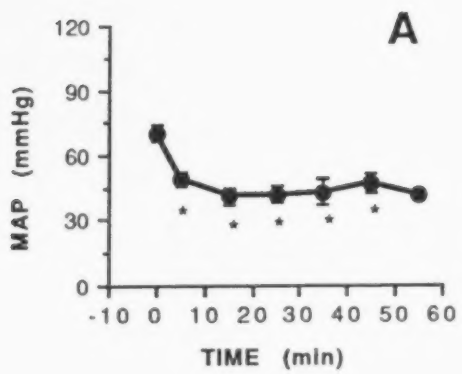
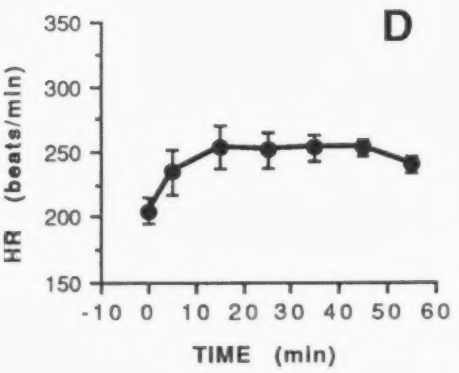
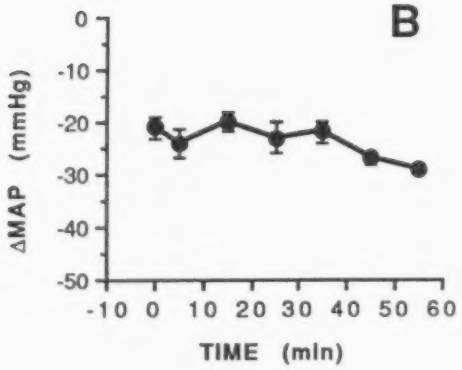
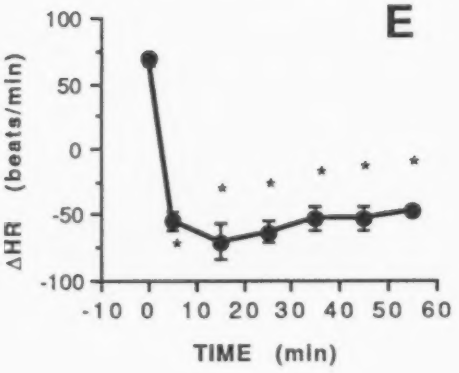

F
t1/2 RECOVERY (s)
100
80
60
40
20
0
-10 0 10 20 30 40 50 60
TIME (min)

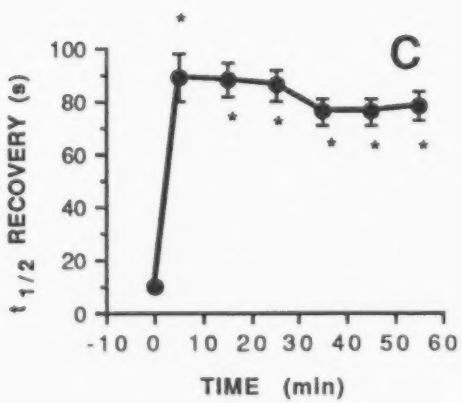
Figure 14: Dilazep 0.5 $mg.kg^{-1}$ (0.8 $\mu mole.kg^{-1}$) time-course in conscious rabbits. The changes in the magnitude of the mean arterial pressure (MAP, mmHg, ordinate, A) and heart rate (HR, $beats.min^{-1}$, ordinate, D) with time (min, abscissae) following intravenous administration of a single dose of dilazep are shown. Also, changes in the magnitude of MAP (B) and HR (E) responses and the $t_{1/2}$ recovery times (s) of the MAP (C) and HR (F) responses to adenosine at various times following dilazep administration are presented. Data points represent the mean ± SEM of 5 different experiments. Asterisks indicate points that are significantly different ($P< 0.05$) from pre-drug baseline values.


A
MAP (mmHg)
120
90
60
30
0
-10 0 10 20 30 40 50 60
TIME (min)


D
HR (beats/min)
350
300
250
200
150
-10 0 10 20 30 40 50 60
TIME (min)


B
ΔMAP (mmHg)
0
-10
-20
-30
-40
-50
-10 0 10 20 30 40 50 60
TIME (min)


E
ΔHR (beats/min)
100
50
0
-50
-100
-10 0 10 20 30 40 50 60
TIME (min)


C
t 1/2 RECOVERY (s)
100
80
60
40
20
0
-10 0 10 20 30 40 50 60
TIME (min)

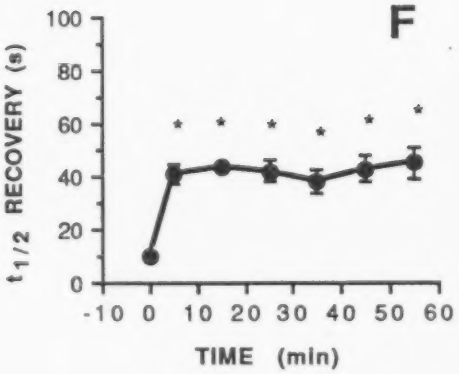
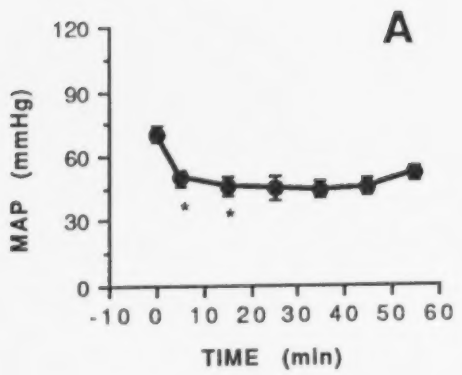
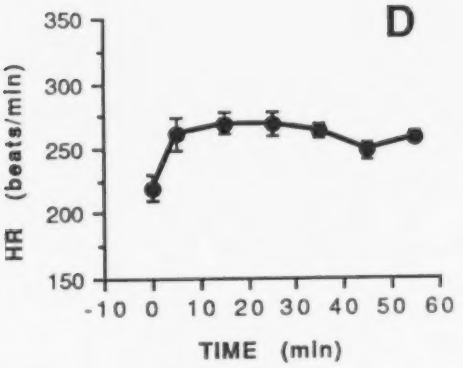
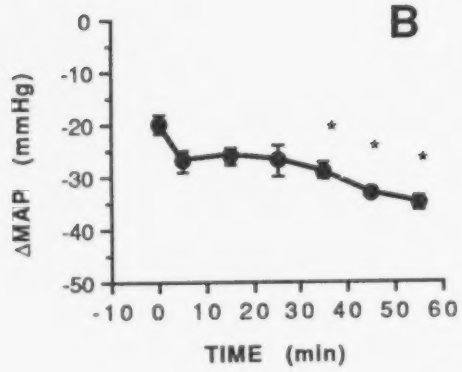
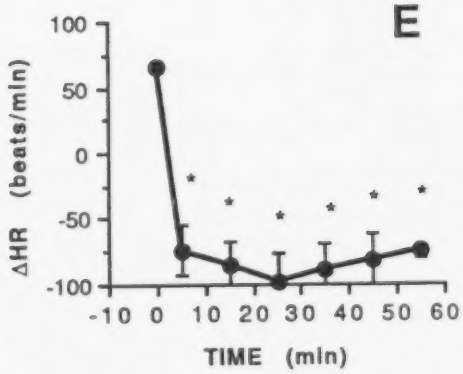

F
t 1/2 RECOVERY (s)
100
80
60
40
20
0
-10 0 10 20 30 40 50 60
TIME (min)

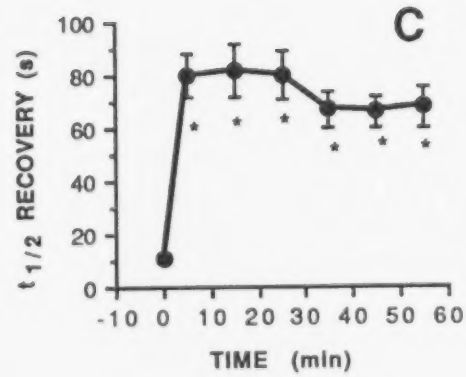
Figure 15: NBMPR-P 1 $mg.kg^{-1}$ (1.8 $\mu mole.kg^{-1}$) time course in conscious rabbits. The changes in the magnitude of the mean arterial pressure (MAP, mmHg, ordinate, A) and heart rate (HR, $beats.min^{-1}$, ordinate, D) with time (min, abscissae) following intravenous administration of a single dose of NBMPR-P are shown. Also, changes in the magnitude of MAP (B) and HR (E) responses and the $t_{1/2}$ recovery times (s) of the MAP (C) and HR (F) responses to adenosine at various times following NBMPR-P administration are presented. Data points represent the mean ± SEM of 5 different experiments. Asterisks indicate points that are significantly different ($P< 0.05$) from pre-drug baseline values.


A
MAP (mmHg)
120
90
60
30
0
-10 0 10 20 30 40 50 60
TIME (min)


D
HR (beats/min)
350
300
250
200
150
-10 0 10 20 30 40 50 60
TIME (min)


B
ΔMAP (mmHg)
0
-10
-20
-30
-40
-50
-10 0 10 20 30 40 50 60
TIME (min)


E
ΔHR (beats/min)
100
50
0
-50
-100
-10 0 10 20 30 40 50 60
TIME (min)


C
t 1/2 RECOVERY (s)
100
80
60
40
20
0
-10 0 10 20 30 40 50 60
TIME (min)

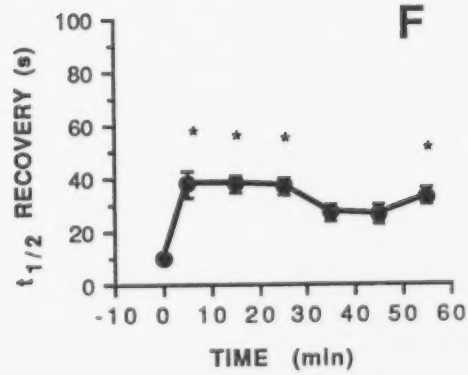
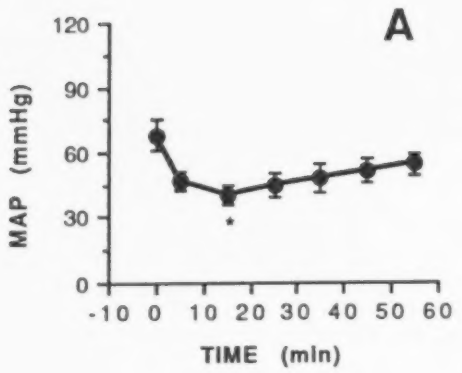
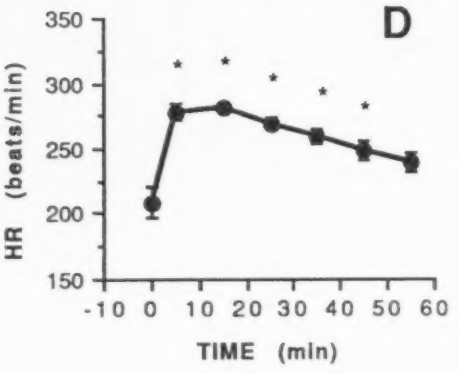
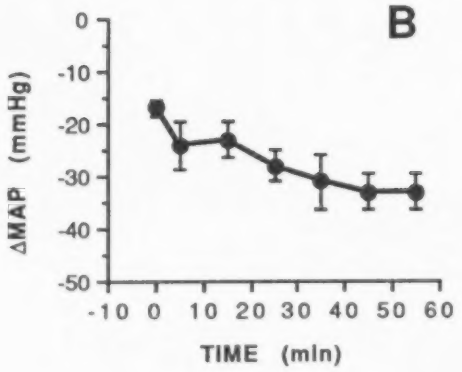
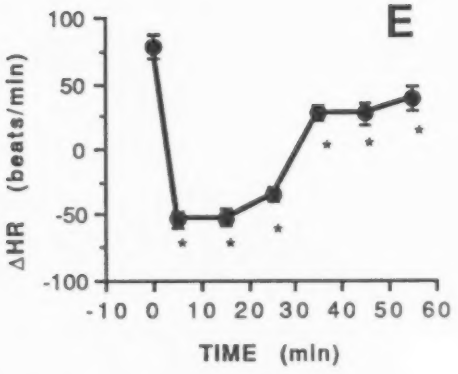

F
t 1/2 RECOVERY (s)
100
80
60
40
20
0
-10 0 10 20 30 40 50 60
TIME (min)

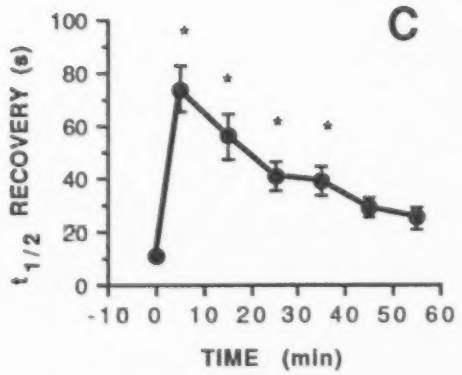
Figure 16: NBTGR-P 1 $mg.kg^{-1}$ (1.7 $\mu mole.kg^{-1}$) time-course in conscious rabbits. The changes in the magnitude of the mean arterial pressure (MAP, mmHg, ordinate, A) and heart rate (HR, $beats.min^{-1}$, ordinate, D) with time (min, abscissae) following intravenous administration of a single dose of NBTGR-P are shown. Also, changes in the magnitude of MAP (B) and HR (E) responses and the $t_{1/2}$ recovery times (s) of MAP (C) and HR (F) responses to adenosine at various times following NBTGR-P administration are presented. Data points represent the mean ± SEM of 6 different experiments. Asterisks indicate points that are significantly different ($P< 0.05$) from pre-drug baseline values.


A
MAP (mmHg)
120
90
60
30
0
-10 0 10 20 30 40 50 60
TIME (min)


D
HR (beats/min)
350
300
250
200
150
-10 0 10 20 30 40 50 60
TIME (min)


B
ΔMAP (mmHg)
0
-10
-20
-30
-40
-50
-10 0 10 20 30 40 50 60
TIME (min)


E
ΔHR (beats/min)
100
50
0
-50
-100
-10 0 10 20 30 40 50 60
TIME (min)


C
t1/2 RECOVERY (s)
100
80
60
40
20
0
-10 0 10 20 30 40 50 60
TIME (min)

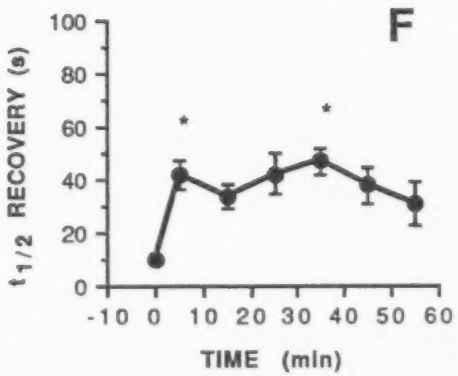
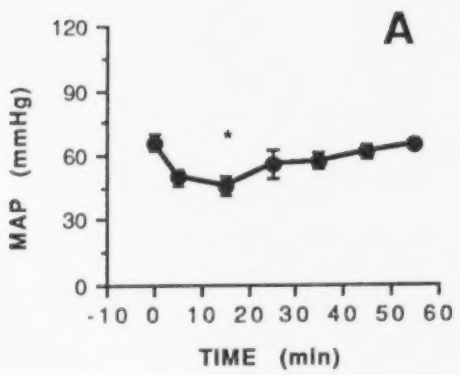
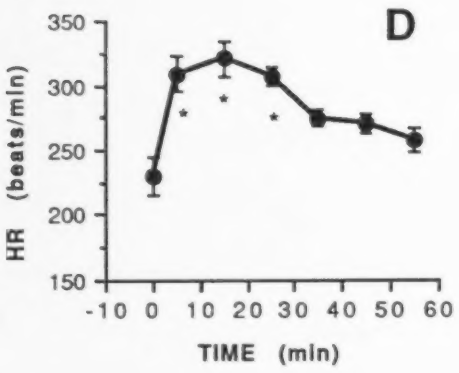
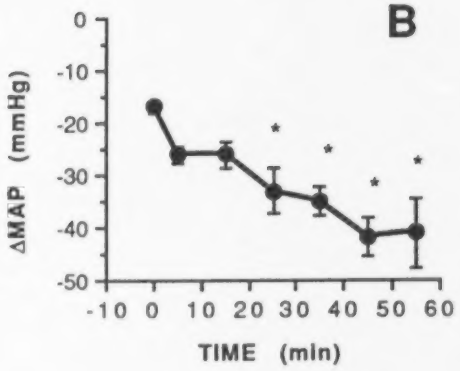
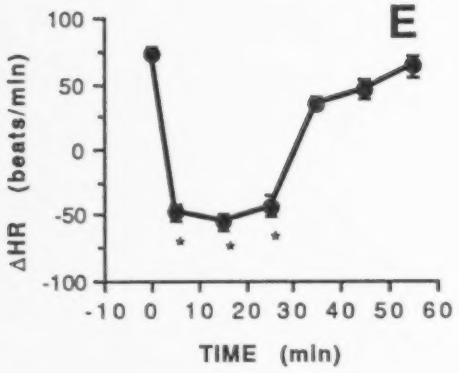

F
t1/2 RECOVERY (s)
100
80
60
40
20
0
-10 0 10 20 30 40 50 60
TIME (min)

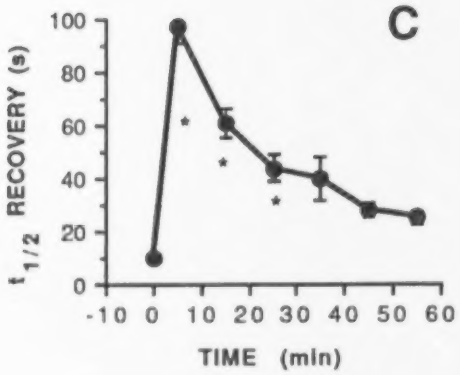
Figure 17: NBdAdo-P 1 $mg.kg^{-1}$ (1.9 $\mu mole.kg^{-1}$) time-course in conscious rabbits. The changes in the magnitude of the mean arterial pressure (MAP, mmHg, ordinate, A) and heart rate (HR, $beats.min^{-1}$, ordinate, D) with time (min, abscissae) following intravenous administration of a single dose of NBdAdo-P are shown. Also, changes in the magnitude of MAP (B) and HR (E) responses and the $t_{1/2}$ recovery times (s) of the MAP (C) and HR (F) responses to adenosine at various times following NBdAdo-P administration are presented. Data points represent the mean ± SEM of 5 different experiments. Asterisks indicate points that are significantly different ($P< 0.05$) from pre-drug baseline values.


A
MAP (mmHg)
120
90
60
30
0
-10 0 10 20 30 40 50 60
TIME (min)


D
HR (beats/min)
350
300
250
200
150
-10 0 10 20 30 40 50 60
TIME (min)


B
ΔMAP (mmHg)
0
-10
-20
-30
-40
-50
-10 0 10 20 30 40 50 60
TIME (min)


E
ΔHR (beats/min)
100
50
0
-50
-100
-10 0 10 20 30 40 50 60
TIME (min)


C
t1/2 RECOVERY (s)
100
80
60
40
20
0
-10 0 10 20 30 40 50 60
TIME (min)

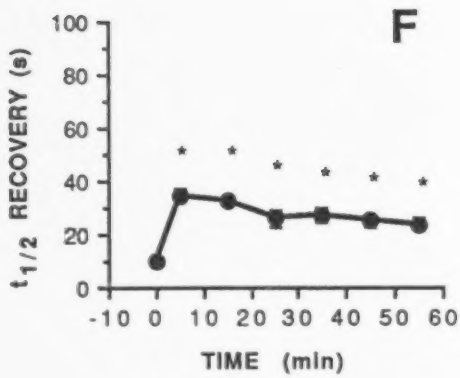
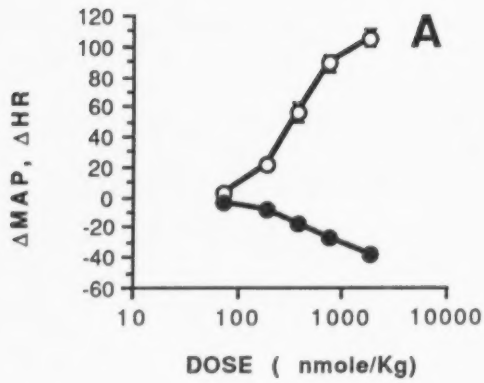
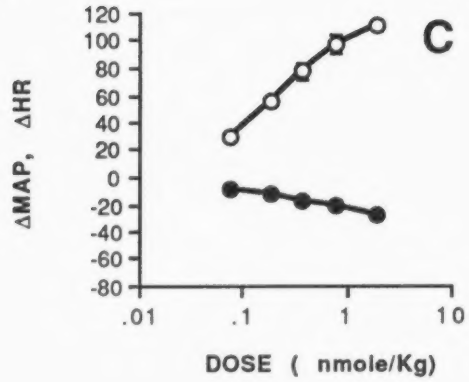
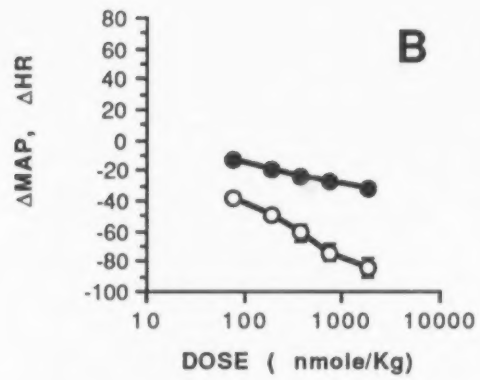

F
t1/2 RECOVERY (s)
100
80
60
40
20
0
-10 0 10 20 30 40 50 60
TIME (min)

Figure 18: Arterial pressure (solid symbols) and heart rate (open symbols) responses to different doses of adenosine (A, B) and sodium nitroprusside (C, D) in the absence (A, C) and presence (B, D) of NBMPR-P in conscious rabbits. The changes of mean arterial pressure (ΔMAP, mmHg, ordinate) and the changes of heart rate (ΔHR, beats. min^{-1}, ordinate) following rapid intravenous administration of different doses of adenosine (A, B, abscissae) and sodium nitroprusside (C, D, abscissae) are shown. Data points represent the mean ± SEM of 4 different experiments.


A
ΔMAP, ΔHR
120
100
80
60
40
20
0
-20
-40
-60
10
100
1000
10000
DOSE (nmole/Kg)


C
ΔMAP, ΔHR
120
100
80
60
40
20
0
-20
-40
-60
-80
.01
.1
1
10
DOSE (nmole/Kg)


B
ΔMAP, ΔHR
80
60
40
20
0
-20
-40
-60
-80
-100
10
100
1000
10000
DOSE (nmole/Kg)

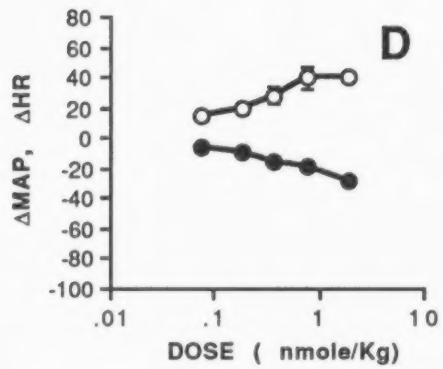

D
ΔMAP, ΔHR
80
60
40
20
0
-20
-40
-60
-80
-100
.01
.1
1
10
DOSE (nmole/Kg)

Figure 19: Relationship between adenosine- (ADO) and sodium nitroprusside- (SNP) induced changes in MAP and HR in conscious rabbits: The changes in heart rate (HR, beats.min^{-1}, ordinate) as a function of changes in mean arterial pressure (MAP, mmHg, abscissae) following different doses of adenosine (■ □) and SNP (● ○) in the absence (solid symbol) and presence (open symbol) of NBMPR-P are shown. Data points represents the mean ± SEM of 4 different experiments.

ADO and SNP RESPONSES

MAP - HR RELATIONSHIPS

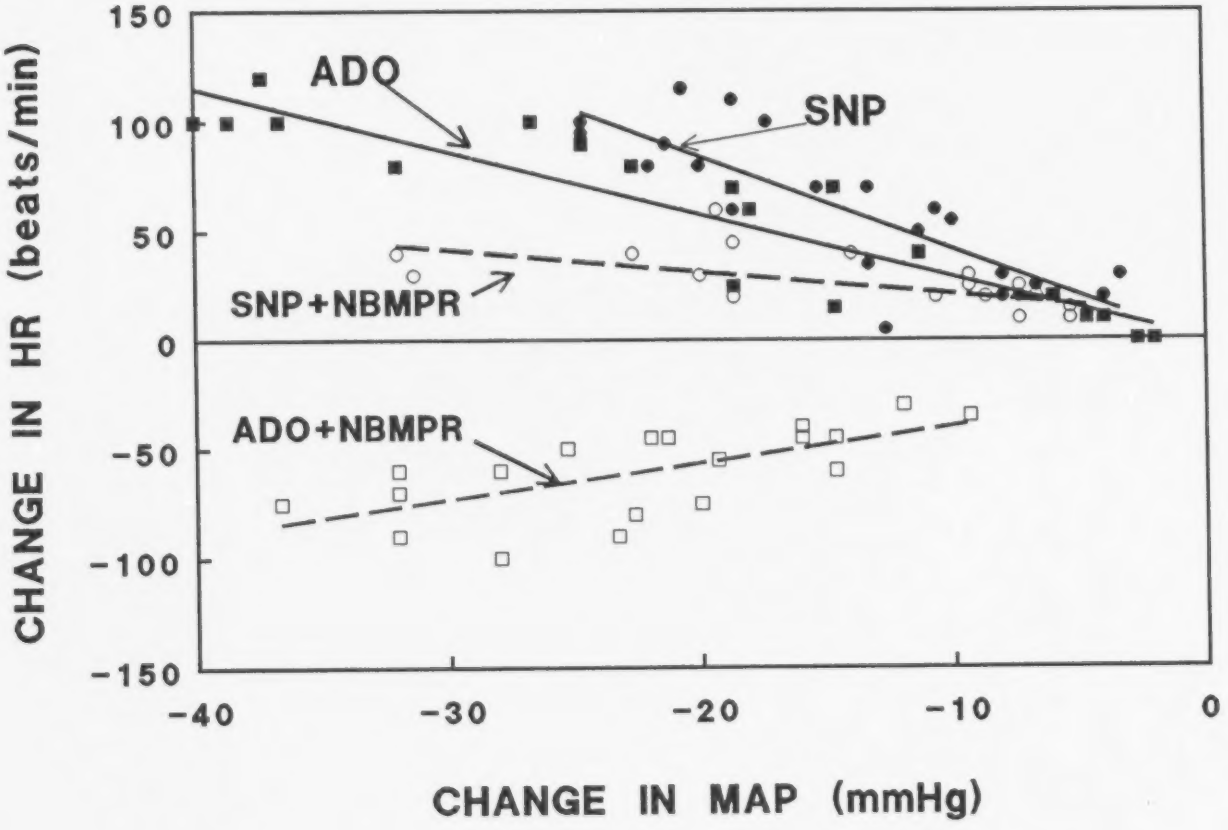


Figure 20: Effects of various receptor antagonists on adenosine-induced hypotension in conscious rabbits. Responses to equi-effective hypotensive dosages of adenosine (A), SNP (S), histamine (H) and INA (I) in the presence of cumulative administration of diphenhydramine and cimetidine, propranolol and then 8-sulfophenyltheophylline (8-SPT) are shown and are expressed as percentage changes from control values. Values shown represent the mean ± SEM for 5 different experiments. Asterisks indicate values that are significantly different from control.

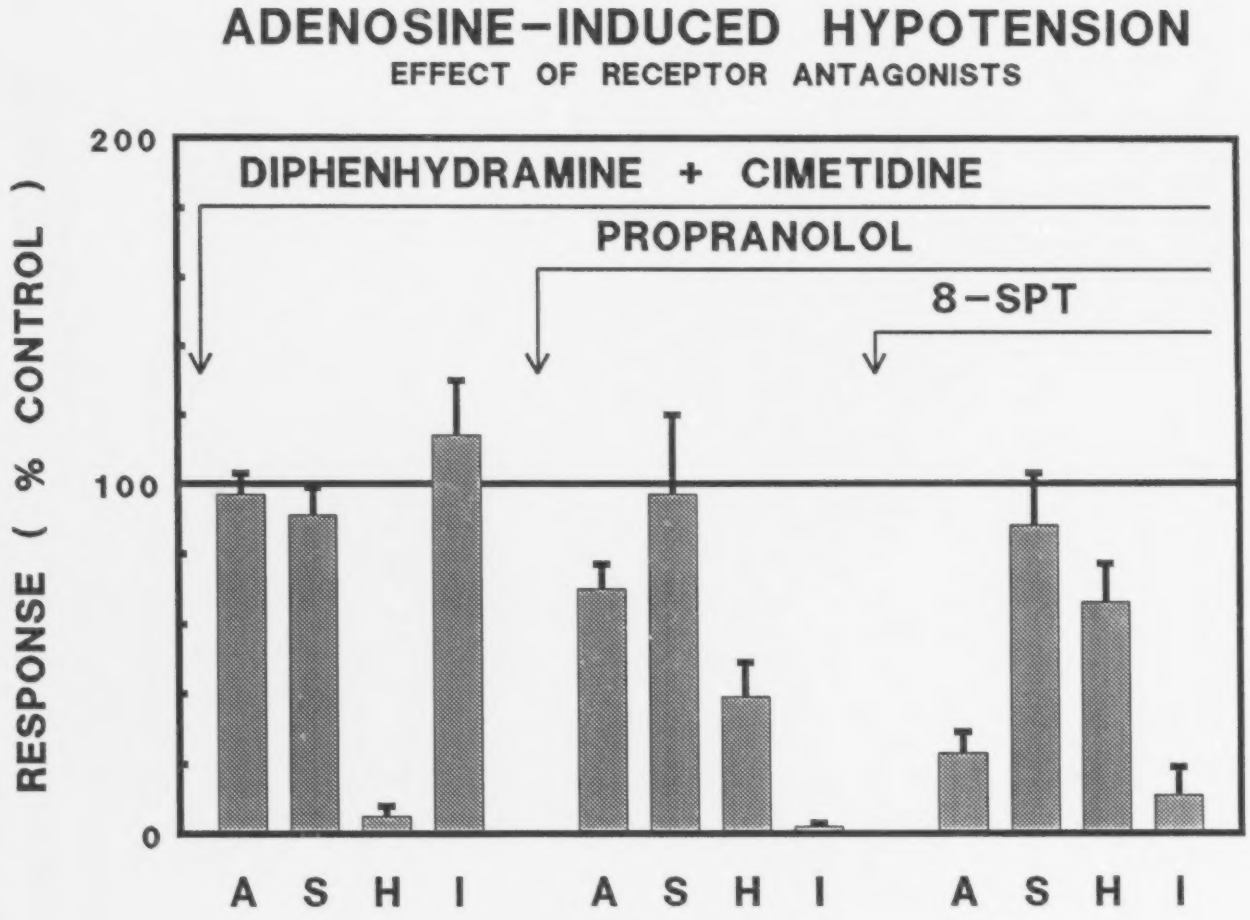
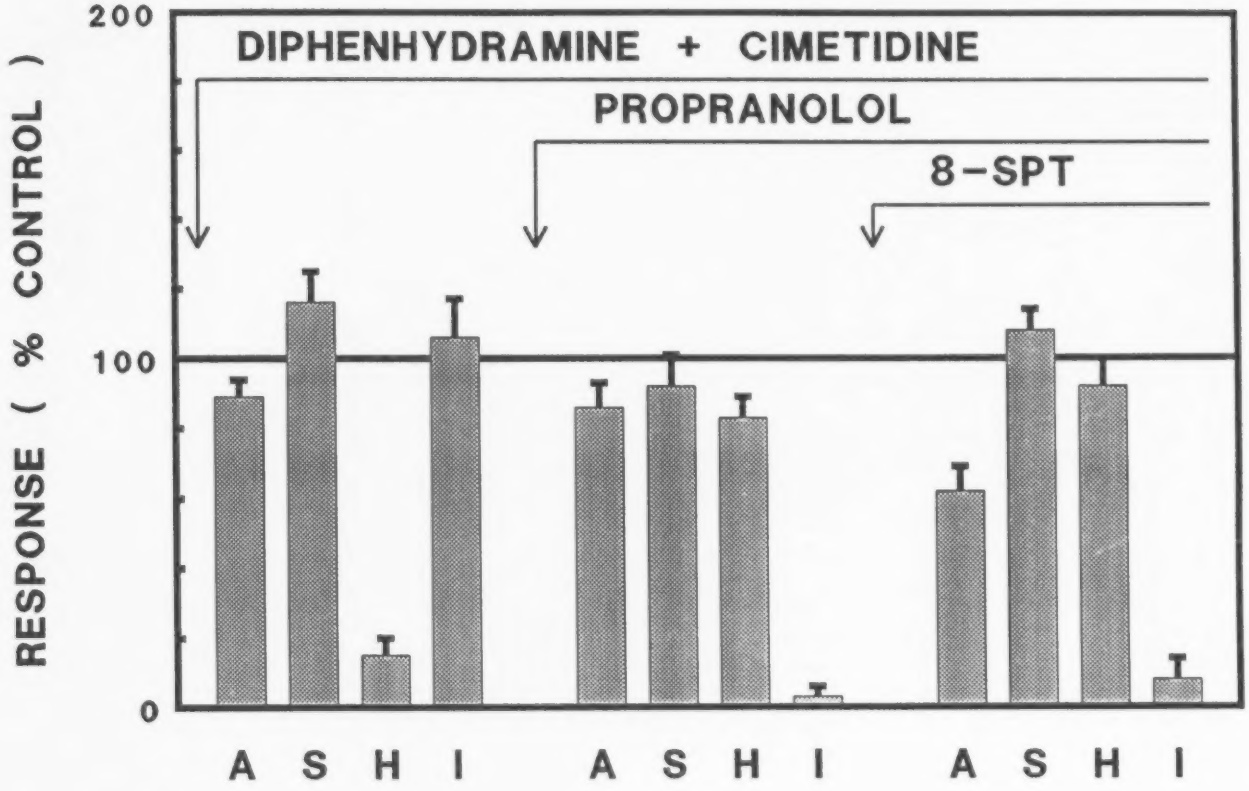

ADENOSINE-INDUCED HYPOTENSION
EFFECT OF RECEPTOR ANTAGONISTS
RESPONSE (% CONTROL)
200
100
0
DIPHENHYDRAMINE + CIMETIDINE
PROPRANOLOL
8-SPT
A S H I A S H I A S H I

Figure 21: Effects of various receptor antagonists on adenosine-induced tachycardia in conscious rabbits. Responses to equi-effective hypotensive dosages of adenosine (A), SNP (S), histamine (H) and INA (I) in the presence of cumulative administration of diphenhydramine and cimetidine, propranolol and then 8-sulfophenyltheophylline (8-SPT) are shown and are expressed as percentage changes from control values. Values shown represent the mean ± SEM for 5 different experiments. Asterisks indicate values that are significantly different from control.

ADENOSINE-INDUCED TACHYCARDIA

EFFECT OF RECEPTOR ANTAGONISTS



IV DISCUSSION

A. Effects of adenosine on arterial pressure and HR

The marked hypotensive effect of intravenously administered adenosine, that was observed in both anaesthetized rats and conscious rabbits, was most likely due to its well documented relaxant effect on the systemic vasculature (Herlihy et al., 1976; Sollevi et al., 1984) that arises from adenosine A_2 receptor stimulation (Collis and Brown, 1983; Webb et al., 1990). Additional depression of arterial pressure may have arisen from a reduction in cardiac output due to its negative inotropic actions on cardiac muscle (Belardinelli et al., 1989). The sensitivities of both species, as assessed by ED_{15} values, were different (135 ± 17 $\mu mole.kg^{-1}$ and 292 ± 84 $\mu mole.kg^{-1}$ $P<0.01$, for rats and rabbits, respectively). The lower sensitivity of rabbits to adenosine may be related to a greater rate of removal of adenosine from the circulation in the rabbit as a consequence of the significantly higher abundance of nucleoside transporter elements on rabbit erythrocytes relative to those of rat (Jarvis et al., 1982b).

The effects of adenosine on HR are more complex. While most studies in anaesthetized animals have reported adenosine-induced bradycardia (Drury and Szent-Gyorgyi, 1929; Jonzon et al., 1986), adenosine-induced tachycardia is observed in conscious human subjects (Biaggioni et al., 1986; Biaggioni et al., 1987) and in conscious animals (Hintze et al., 1985; Fukunaga et al., 1982). It is more likely that the different HR responses are due to anaesthesia rather than to species differences because in this study adenosine-induced tachycardia was also observed in conscious rabbits. The net effect of adenosine on HR depends on the balance between direct and indirect effects. Adenosine has a direct negative chronotropic effect that is induced by adenosine A_1 receptors that cause direct depression of SA nodal function and

which is clearly demonstrable *in vitro* (Belardinelli and Isenberg, 1983). However, *in vivo*, indirect reflex actions may also alter HR. The marked hypotension elicited by adenosine would, via the baroreceptor reflex, cause withdrawal of vagal tone (Belloni et al.,1989) and an increase in sympathetic tone to the heart thereby tending to increase HR. A reflexly-mediated increase in HR in association with hypotension is seen in conscious preparations (Biaggioni et al., 1986). In some types of anaesthetized preparations, this baroreceptor reflex response is attenuated (Fukunaga et al., 1982) and the direct SA nodal depressant effect predominates. In other anaesthetized preparations, using combinations of anaesthetics that "reset" autonomic tone and HR towards control levels, adenosine, and other vasodilators, then elicit tachycardia in response to hypotension (Finegan et al., 1990). Adenosine-induced tachycardia is, however, less than that observed with equi-effective hypotensive doses of other vasodilators such as glyceryl trinitrate (Hintze et al., 1985) or sodium nitroprusside (Finegan et al. 1990), a response due to some degree of direct SA nodal depression by adenosine.

The duration of action of adenosine in both species was extremely short. The $t_{1/2}$ recovery times, that were dose-dependent, ranged from 5 ± 1 to 78 ± 12 s in rats and 2 ± 1 to 20 ± 2 s in rabbits. At equi-effective hypotensive dosages (15 mmHg), the duration of the hypotensive responses were not significantly different between species (8 ± 1 and 6 ± 1 s in rats and rabbits, respectively). Similarly, for the HR responses, $t_{1/2}$ recovery times, despite the difference in direction, were rapid and were not significantly different between species at equi-effective hypotensive doses (7 ± 1 and 7 ± 1 s in rats and rabbits, respectively). The short duration of adenosine action reflects the efficient mechanisms that control adenosine levels in the circulation. These include the

mediated passage of adenosine across cell membranes by NT systems (Paterson et al., 1983; Gati and Paterson, 1989) followed by its intracellular metabolism by adenosine kinase (Berne, 1986) and adenosine deaminase (Berne, 1986; Geiger and Nagy, 1990). Circulatory erythrocytes are considered to be an important sink of adenosine in the circulation and the marked differences between plasma elimination rate constants for adenosine in man and dog (Moser et al., 1989) can be explained by the marked differences in the erythrocytic abundance of the NBMPR-sensitive facilitated nucleoside transporter (Jarvis et al., 1982b). The similarity between adenosine inactivation, as judged by a functional criterion (recovery $t_{1/2}$), in rabbit and rat, despite marked differences in their erythrocytic abundance of NBMPR-sensitive NT systems (Jarvis et al., 1982b), suggests that other mechanisms and/or cell types must also participate in the regulation of adenosine levels in the circulation.

B. Effects of NTIs on arterial pressure and HR

The dose-dependent reduction in arterial pressure in anaesthetized rats and in conscious rabbits caused by the NTIs indicated a systemic vasodilation effect of all the transport inhibitors used in this study. Information on the hemodynamic actions of NBMPR-P, NBTGR-P and NBdAdo-P *in vivo* is not available in the literature and this study is the first to show their effects on arterial pressure and HR in animals. Up to this point, the literature suggests that the vasodilation elicited by other NTIs may be due to adenosine. By blocking adenosine uptake into cells, the transport inhibitors potentiate the cardiovascular effects of endogenous adenosine. These include vasodilation through adenosine A_2 receptors on vascular endothelial cells (Nees et al., 1987) and smooth muscle cells that leads to a decrease in systemic vascular resistance and a cardiac inhibitory effect through adenosine A_1 receptor on the

heart that leads to a decrease in cardiac output. The support of this hypothesis is based on studies with dipyridamole, dilazep and mioflazine. Dipyridamole is a renal vasoconstrictor (Arend et al., 1985) as well as a coronary vasodilator. Based on the fact that both the coronary dilation and renal constriction effects of dipyridamole are reversed by adenosine receptor antagonists (Afonso, 1970; Sollevi et. al. 1984; Arend et al., 1985), it has been concluded that these effects of dipyridamole are mediated by potentiating the effects of endogenous adenosine. Some other studies also demonstrated that the vasodilatory effects of dilazep, dipyridamole (Kalsner, 1975; Mustafa, 1979) and mioflazine (Buchwald et al., 1987) are achieved through the potentiation of adenosine. Interestingly, the order of affinities of NTIs for NBMPR binding sites (K_d values) is not related to their hypotensive effects (Table 2). Since the affinities of NTIs for NBMPR binding sites are considered to be representative of their ability to inhibit one type of NT (Hammond et al., 1981; Clanachan et al., 1987), the lack of correlation between the hypotensive effects of the NTIs and their affinities for NBMPR binding sites may indicate that separate mechanisms are responsible for their hypotensive and NT inhibitory effects.

A second possible mechanism for the hypotensive effect of NTIs is through the inhibition of calcium influx. It has been demonstrated that dilazep possesses definite calcium antagonist properties (Tonini et al., 1983; Nakagawa et al., 1986). It has also been shown that dilazep and lidoflazine produce a direct relaxant effect on isolated dog cerebral and renal arteries through their calcium entry blocking effects which are independent of adenosine (Mustafa and Nakagawa, 1988). This may explain the more potent hypotensive effect of dilazep relative to other NTIs. The fact that both dilazep and dipyridamole have equipotent adenosine uptake inhibitory effects (Mustafa,

Table 2. Comparison of the relative potencies of dipyridamole, dilazep, NBMPR-P, NBTGR-P and NBdAdo-P.

	DIP	DIL	NBMPR-P	NBTGR-P	NBdAdo-P
K_d or K_i (nM)	1700	34	0.4	0.4	NA
IC_{50} Influx (nM)		8	1.7	0.1	4
IC_{50} Binding (nM)	10	2	0.31	2.5	NA
Hypotension	37±8.8	0.7±0.1	9.5±2.7	8.9±1.8	44±11.3
Prolongation	35±14	1.4±0.4	4.1±1.3	1.8 ± 0.3	3.2 ±0.9
Selectivity Index	2.5±1.1	0.9±0.3	2.8±0.8	5.4±1.6	14.7±3.1
Relative Index	2.8	1	4.7	6.0	16.3

Literature values are shown for the potencies of the NTIs as measured by their affinity (Kd/Ki) for [3H]NBMPR binding sites on rat cardiac tissues (Clanachan et al., 1987), their ability to inhibit adenosine influx (IC_{50} Influx) in S49 cells grown in culture (Paterson et al., 1983) and by their ability to inhibit the site-specific binding (IC_{50} Binding) to human erythrocytes (Cass et al., 1974). NA- data not availlable. The doses (μmole.kg-1) required to reduce MAP by 15 mmHg (Hypotension) and to cause adenosine potentiation as determined by a two fold prolongation of adenosine-induced hypotension (Prolongation) in rats are also shown. A Selectivity Index for each NTI, calculated as the ratio of the hypotension and prolongation dosages, is also presented. The values shown for the Relative Index are based on the Selectivity Index but normalized, based on a value for dilazep of 1. Values for Hypotension, Prolongation and Selectivity Index were calculated from dose-response curves for individual animals and results are presented as mean values ± SEM for at least six animals in each drug group. The Selectivity Index for dilazep was significantly lower than for the other drugs, while the greatest Selectivity Index was found with NBdAdo-P.

1979), while dilazep, but not dipyridamole, has been shown to have a calcium entry blocking effect which may responsible for its vasodilatory effects (Nakagawa et al., 1986) also suggests that the degree of hypotensive action of NTIs may not solely be related to their transport inhibitory effects.

Another component of the vasodilatory effect of NTIs may be due to phosphodiesterase inhibition or to adenosine receptor activation. It has been demonstrated that the vasodilatory and antiplatelet effects of dipyridamole are achieved through both NT inhibition and phosphodiesterase inhibition (Dawicki et al., 1985). Also, binding studies have shown that some NTIs have affinity for adenosine receptors (Lohse et al., 1985; Michaelis et al., 1985) and so could cause vasodilation by adenosine receptor activation.

Thus, the results of this study indicate a lack of relationship between the hypotensive and the transport inhibitory effects of NTIs (Table 2) in the rat. There is no correlation between transport inhibition by the NTIs, as judged by K_d or K_i values, and the hypotensive abilities of the NTIs as judged by equi-effective hypotensive doses (ED_{15}) (Table 2). The data in Table 2 also indicates that, by using different methods to measure the affinity of NTIs for NT systems, one can obtain different order of potencies. Therefore, NTI-induced prolongation of adenosine action ($t_{1/2}$) , as used in this study, is probably a better functional index of the ability of NTIs to alter nucleoside disposition *in vivo*.

All the NTIs, except NBdAdo-P, had a significant hypotensive effect in the rat. Nevertheless, NBdAdo-P still caused a significant potentiation of adenosine-induced actions. This strongly suggests that the NT inhibitory effect and the hypotensive effect of NTIs are separable. The order of potency of NTIs on blood pressure in rat is as follows: dilazep > NBTGR-P = NBMPR-P >

dipyridamole > NBdAdo-P. Although all the NTIs, except dipyridamole, caused hypotension in rabbits, their prolongation of adenosine-induced hypotension was longer lasting than their hypotensive effects. This again suggests that the hypotensive and NT inhibitory effects of the NTIs are separable.

The opposite effects of NTIs on HR (bradycardia in anaesthetized rats and tachycardia in conscious rabbits) can be explained in the same manner as for adenosine. The tachycardia in conscious rabbits is probably due to the reflexly increased sympathetic tone that accompanies hypotension. In contrast, in anaesthetized rats, no reflex tachycardia is evident despite a similar degree of drug-induced hypotension. Only bradycardia was observed in rats and is probably due to either direct SA nodal depression or to a potentiation of the effect of endogenous adenosine on A_1 receptors in the SA node (Fukunaga et al., 1982). In rats, only dilazep caused significant decreases in HR, while in rabbits, only NBTGR-P and NBdAdo-P caused significant increases in HR. The significant decrease in HR that was caused by dilazep is probably due to its Ca^{2+} entry blocking effect.

Arterial pressure and HR in rabbit were more sensitive to NTI-induced alterations than in rat. This species difference is probably due to the lower sensitivity of rat tissues to the NTIs (Hopkins and Goldie, 1971). Also, NBMPR binding studies have shown that rat tissues tend to possess a significant lower number of NT inhibitory sites (Hammond and Clanachan, 1985).

C. Effects of NTIs on adenosine responses

The two indices of the interactions of NTIs and adenosine that have been used in this study are potentiation of, (1) the magnitude and (2) the duration of adenosine-induced alterations in MAP and HR. It was demonstrated that all of

the NTIs tested were able to potentiate adenosine responses, effects likely arising from their ability to inhibit NT and hence the inactivation of adenosine in the circulation. Interestingly, NTI-induced potentiation of the magnitude of adenosine effects was observed only with NBdAdo-P, whereas potentiation, as measured by the prolongation of adenosine responses, was demonstrated for all NTIs tested.

The inability of the NTIs (except NBdAdo-P) in rat to potentiate the magnitude of adenosine-induced hypotension is at first surprising since there is an extensive literature on drugs such as dipyridamole (Kolassa et al., 1970; Williams et al., 1984; Biaggioni et al., 1986) and dilazep (Mustafa, 1979). It should be noted, however, that in this study the dose of NTIs used were hypotensive *per se*. Thus, in rats, the magnitudes of the hypotensive effect of adenosine *in vivo* were not comparable between pre-NTI conditions and post-NTI when baseline MAP had already been depressed. In support, NBdAdo-P, which had the least hypotensive action *per se*, did indeed potentiate the magnitude of adenosine-induced hypotension. The magnitude of HR responses to adenosine were also not potentiated despite relatively minor NTI-induced changes in baseline values. This resistance of adenosine-induced HR responses in the rat to alteration by NTIs suggests that NT may not be involved in the control of this effect of adenosine. HR responses to adenosine are of shorter duration and are probably mediated by the initial high concentration of adenosine passing through the heart following intravenous administration. This local high concentration dissipates rapidly due to the high blood flow thus rendering local inactivation mechanisms for adenosine less important in the control of the intensity of the response. Unexpectently, dilazep attenuated, rather than potentiated, the bradycardic effect of adenosine. This response was not observed with the other NTIs and probably is due to the marked direct

effects of dilazep on HR. Dilazep depressed HR so that further depression by adenosine was not possible.

Potentiation of adenosine effects by NTIs was more easily observed as a prolongation of the duration of action of adenosine, either on MAP or on HR, rather than as a potentiation of the magnitude of adenosine action. Comparisons of the calculated doses of each of the NTIs that would cause a two-fold increase in the duration of action of adenosine showed the following order of potency: dilazep = NBTGR-P > NBdAdo-p = NBMPR-P > dipyridamole. This is different from their order of potency as hypotensive agents. Calculation of the ratio of doses (see Table 2) required for hypotension (15 mmHg change) and for prolongation (2 fold), revealed that significant differences exist among NTIs. Of particular importance is the demonstration that the hypotension/prolongation ratio is largest for NBdAdo-P (14.7 ± 3.1) indicating that potentiation of the duration of action of adenosine can be obtained at doses that are virtually devoid of hypotensive activity. This result has important implications for the further development of NTIs as therapeutic agents, either as indirect adenosinemimetics or in host protection strategies in chemotherapy. It seems that adenosine potentiation, that can be obtained with NTIs, need not be accompanied by undesirable hypotensive actions.

NTI-adenosine interactions in rabbits were examined only at a single dose-level of each NTI. Consequently, hypotension/prolongation ratios could not be calculated. Nevertheless, from the time-course studies in rabbits, a similar dissociation between the direct hypotensive effects of NTIs and their ability to potentiate adenosine was observed. Significant potentiation of adenosine responses was observed at times followed NTI administration when no alterations in arterial pressure were demonstrable. NTIs were more

efficacious in rabbits than in rats. At the dose and time points that can be compared, all NTIs elicited greater responses on MAP, HR, $t_{1/2}$ in the rabbit than in the rat. This species difference may reflect the greater abundance of NT sites on the erythrocytes in the rabbit circulation (Jarvis et al., 1982b). In rats, only NBdAdo-P potentiated the magnitude of adenosine-induced hypotensive responses, again probably due to the direct depressant effect of the other NTIs on arterial pressure. Potentiation of adenosine was more readily demonstrated as a prolongation of the duration of the arterial response. Examination of the time-courses of the prolongation of the adenosine hypotensive response reveals differences in the duration of action of the NTIs. Prolongation was best with dilazep and NBMPR-P (up to 60 min after administration) whereas with dipyridamole, NBTGR-P and NBdAdo-P, maximum prolongations were 5 min. This was followed by a fairly rapid recovery in the next 30-40 min. These difference suggest important pharmacokinetic differences among these NTIs. Potential pharmacokinetic differences deserve further evaluation and may ultimately influence the choice of NTI for the various potential clinical indications.

Special consideration must be given to the effects of the NTIs on adenosine-induced alterations in HR in conscious rabbits. As was discussed above, adenosine elicited a dose-dependent tachycardia, probably due to a reflex increase in sympathetic tone that accompanies hypotension. Although the NTIs had caused minimal effects on baseline HR and no marked changes in the magnitude of the adenosine-induced hypotension (the actual stimulus for the tachycardia), they reversed the effect of adenosine on HR from a tachycardia to a bradycardia. The duration of the reversal depended on the pharmacokinetics of the individual NTI. In the presence of the longer acting

agents, eg. dilazep and NBMPR-P, the tachycardia was reversed for greater than 60 min, whereas with the other NTIs, the reversal lasted for 10 to 20 min and then the tachycardia in response to adenosine returned to control levels. In order to determine if the reversal of vasodilator-induced tachycardia was specific for adenosine, a second vasodilator, SNP, was used. With doses of SNP that caused similar, dose-dependent, hypotensive responses, similar reflex increases in HR were observed. After the administration of the NTI, NBMPR-P, the adenosine-induced tachycardia was reversed to a bradycardia, whereas reflex tachycardia in response to SNP was still demonstrable. This occurred even although arterial pressure was reduced to approximately the same levels by both adenosine and SNP. Thus, the reversal of vasodilator-induced tachycardia to bradycardia is specific to adenosine, rather than to an effect of NBMPR-P on baroreceptor mechanisms or cardiovascular control centres in the CNS. The underlying mechanism may be related to the dual effects of adenosine on HR: direct SA nodal depression and indirect activation via baroreceptor stimulation in response to hypotension. In the absence of an NTI, reflex sympathetic activation overcomes the direct depressant effects of adenosine and a tachycardia is observed. After the NTI, the reflex sympathetic activation, that remains the same because the hypotension is unchanged by the NTI, is overcome by the now concentrated and therefore greater direct depressant effects of adenosine on the SA node. This is evidence for NTI-induced potentiation of the direct HR response to adenosine in the rabbit. This is in contrast to results discussed above for the rat, where NTIs did not potentiate the direct depressant actions of adenosine on HR. As for arterial pressure responses, this species difference may be due to the marked differences in the erythrocytes' abundance of NT systems in these two species. The rabbit, with an NBMPR site-density on RBCs closer to that of man (8 to 10 K

sites/cell), appears to be a more appropriate animal for the study of NTI-adenosine interactions.

D. The effects of receptor antagonists on adenosine-induced hypotension and tachycardia

Since adenosine has interactions with sympathetic neurotransmitter and histamine release, the effects of adenosine, SNP, histamine and INA on MAP and HR were investigated in the presence and absence of their respective receptor antagonists in order to determine the mechanism of the effects of adenosine on HR. The results of this study indicated the cardiovascular effects of adenosine are mainly mediated via adenosine receptors.

After administration of a combination of the histamine H_1 and H_2 receptor antagonists, diphenhydramine and cimetidine, the hypotensive and tachycardic effects of histamine were completely inhibited while the effects of adenosine were not influenced (Figs. 20 & 21). This indicated that the effects of adenosine on HR are not likely mediated through interactions with histamine release. The ß-adrenoceptor antagonist, propranolol, at a dose that caused over 95% suppression of the INA-induced changes in MAP and HR, also caused a significant inhibition ($P<0.05$) of adenosine-induced hypotension but not of adenosine-induced HR responses. As the tachycardia in response to the other vasodilator, SNP, was also not affected by propranolol treatment, the data cannot be interpreted to suggest that adenosine was acting independently of reflex sympathetic activation. Rather, it appears that while the dose of propranolol used was effective in blocking the effects of the ß-adrenoceptor agonist, INA, it was unable to block nerve-mediated ß-adrenoceptor activation. After administration of the adenosine receptor antagonist, 8-

sulfophenyltheophylline (8-SPT), the effects of adenosine were greatly suppressed ($P<0.05$). This demonstrated that the main mechanism that is responsible for the cardiovascular effects of adenosine is mediated through adenosine receptors.

E. Heterogeneity of NT

Species differences in the actions of NTIs have long been recognized. In 1971, Hopkins and Goldie found that rat tissue was insensitive to dipyridamole. Subtypes of facilitated diffusion NT systems that have different sensitivities to NBMPR binding have been demonstrated in recent years (Jarvis and Young, 1986; Paterson et al., 1987; Lee and Jarvis, 1988). Facilitated diffusion systems can also subclassified on the basis of sensitivity to dipyridamole (Hammond and Clanachan, 1984; 1985)

There are obvious species differences in the present study. Firstly, for the same fall in BP, the doses of NTIs required are 20 times more in rats than that in rabbits; secondly, the potentiation of adenosine-induced effects by NTIs is more obvious in rabbits than that in rats. The possible reasons for these observations may be related to higher abundance of transporter sites on rabbits erythrocytes relative to those in rat (Jarvis et al., 1982b).

F. Conclusion

The present study has demonstrated a lack of correlation between the hypotensive effects of several NTIs and their NT inhibitory effects (measured either by their binding affinities for NBMPR sites *in vitro* or their potentiation of the duration of the hypotensive effect of adenosine *in vivo*). This suggests that the hypotensive effect of NTIs may not be entirely due to a potentiation of the

vasodilatory effect of endogenous adenosine.

Of all the NTIs tested, NBdAdo-P caused the greatest degree of adenosine potentiation and the least amount of hypotension. This compound should be evaluated further as it may serve as a useful agent to alter the disposition of endogenous or exogenous nucleosides without undesirable hypotensive actions.

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